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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: <b>RECOMBINANT SWINEPOX VIRUS</b>			
(57) Abstract <p>The present invention relates to a recombinant swinepox virus capable of replication comprising foreign DNA inserted into a site in the swinepox viral DNA which is not essential for replication of the swinepox virus. The invention further relates to homology vectors which produce recombinant swinepox viruses by inserting foreign DNA into swinepox viral DNA.</p>			

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RECOMBINANT SWINEPOX VIRUS

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This application is a continuation-in-part of U.S. Serial No. 07/820,154 filed January 13, 1992 and U.S. Serial No. 08/097,554, filed July 22, 1993, the contents of which are incorporated by reference into the present application.

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Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Background of the Invention

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Swinepox virus (SPV) belongs to the family Poxviridae. Viruses belonging to this group are large, double-stranded DNA viruses that characteristically develop in the cytoplasm of the host cell. SPV is the only member of the genus Suipoxvirus. Several features distinguish SPV from other poxviruses. SPV exhibits species specificity (18) compared to other poxviruses such as vaccinia which exhibit a broad host range. SPV infection of tissue culture cell lines also differs dramatically from other poxviruses (24). It has also been demonstrated that SPV does not exhibit antigenic cross-reactivity with vaccinia virus and shows no gross detectable homology at the DNA level with the ortho, lepori, avi or entomopox virus groups (24). Accordingly, what is known and described in the prior art regarding other poxviruses does not pertain a priori to swinepox virus.

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SPV is only mildly pathogenic, being characterized by a self-limiting infection with lesions detected only in the skin and regional lymph nodes. Although the SPV infection is quite limited, pigs which have recovered from SPV are refractory to challenge with SPV, indicating development of active immunity (18).

The present invention concerns the use of SPV as a vector for the delivery of vaccine antigens and therapeutic agents to swine. The following properties of SPV support this rationale: SPV is only mildly pathogenic in swine, SPV is species specific, and SPV elicits a protective immune response. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced against the benefit contributed by the vector's vaccine and therapeutic properties.

The prior art for this invention stems first from the ability to clone and analyze DNA while in bacterial plasmids. The techniques that are available are detailed for the most part in Maniatis et al., 1983 and Sambrook et al., 1989. These publications teach state of the art general recombinant DNA techniques.

Among the poxviruses, five (vaccinia, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. Vaccinia virus has been used extensively to vector foreign genes (25) and is the subject of U.S. Patents 4,603,112 and 4,722,848. Similarly, fowlpox has been used to vector foreign genes and is the subject of several patent applications EPA 0 284 416, PCT WO 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies virus. These examples of insertions of foreign genes into poxviruses do not include an example from the

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genus Suipoxvirus. Thus, they do not teach methods to genetically engineer swinepox viruses, that is, where to make insertions and how to get expression in swinepox virus.

5 The idea of using live viruses as delivery systems for antigens has a very long history going back to the first live virus vaccines. The antigens delivered were not foreign but were naturally expressed by the live virus in the vaccines. The use of viruses to deliver foreign  
10 antigens in the modern sense became obvious with the recombinant vaccinia virus studies. The vaccinia virus was the vector and various antigens from other disease causing viruses were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became  
15 obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate virus vector. In answering this question, details of the pathogenicity of the virus, its site of replication, the kind of immune response it elicits, the potential it has  
20 to express foreign antigens, its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc, are all factors in the selection. The prior art does not teach these questions of utility.

25 The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a vaccinia virus to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the vaccinia vector, the host did not demonstrate any therapeutic  
30 benefit.

The therapeutic agent that is delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the  
35 therapeutic agent in the first analysis to either DNA, RNA

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or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense DNA, anti-sense RNA (16), ribozymes (34), suppressor tRNAs (2), interferon-inducing double stranded RNA and numerous  
5 examples of protein therapeutics, from hormones, e.g., insulin, to lymphokines, e.g., interferons and interleukins, to natural opiates. The discovery of these therapeutic agents and the elucidation of their structure and function  
10 does not make obvious the ability to use them in a viral vector delivery system.

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Summary of the Invention

The invention provides a recombinant swinepox virus capable of replication which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced. The foreign DNA is inserted into the swinepox viral DNA at a site which is not essential for replication of the swinepox virus and is under the control of a promoter.

This invention provides a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus which comprises a double-stranded DNA molecule. This molecule consists essentially of double-stranded foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced. At one end of this foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus. At the other end of the foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA.

Brief Description of the Figures

## Figures 1A and 1B

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show a detailed diagram of SPV genomic DNA (Kasza strain) including the unique long and Terminal repeat (TR) regions. A restriction map for the enzyme *HindIII* is indicated (23). Fragments are lettered in order of decreasing size. Note that the terminal repeats are greater than 2.1 kb but less than 9.7 kb in size.

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## Figures 2A and 2B

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show the DNA sequence from homology vector 515-85.1. The sequence of two regions of the homology vector 515-85.1 are shown. The first region (Figure 2A) (SEQ ID NO:1) covers a 599 base pair sequence which flanks the unique *AccI* site as indicated in Figure 3. The beginning (Met) and end (Val) of a 115 amino acid ORF is indicated by the translation of amino acids below the DNA sequence. The second region (Figure 2B) (SEQ ID NO:3) covers the 899 base pairs upstream of the unique *HindIII* site as indicated in Figure 3. The beginning (Asp) and end (Ile) of a 220 amino acid ORF is indicated by the translation of amino acids below the DNA sequence.

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## Figures 3A, 3B, and 3C

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show the homology which exists between the 515.85.1 ORF and the Vaccinia virus 01L ORF. Figure 3A shows two maps: The first line of Figure 3A is a restriction map of the SPV *HindIII* M fragment and the second is a restriction map of the DNA insertion in plasmid 515-

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85.1. The location of the 515-85.1 [VV 01L-like] ORF is also indicated on the map. The locations of the DNA sequences shown in Figures 3B and 3C are indicated below the map by heavy bars in Figure 3A. Figure 3B shows the homology between the VV 01L ORF (SEQ ID NO:5) and the 515-85.1 ORF (SEQ ID NO:6) at their respective N-termini. Figure 3C shows the homology between the VV 01L ORF (SEQ ID NO:7) and the 515-85.1 ORF (SEQ ID NO:8) at their respective C-termini.

Figures 4A, 4B, and 4D

show a description of the DNA insertion in Homology Vector 520-17.5. Figure 4A contains a diagram showing the orientation of DNA fragments assembled in plasmid 520-17.5 and table indicating the origin of each fragment. Figure 4B shows the sequences located at each of the junctions A and B between fragments, and Figure 4C shows the sequences located at Junctions C and D (SEQ ID NO's: 9, 10, 13, and 16). Figures 4B and 4C further describe the restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements are also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), early promoter 1 (EP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and Escherichia coli (E. coli).

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Figures 5A, 5B, 5C, and 5D

shows a detailed description of the DNA insertion in Homology Vector 538-46.16. Figure 5A contains a diagram showing the orientation of DNA fragments assembled in plasmid 538-46.16 and a table indicating the origin of each fragment. Figure 5B shows the sequences located at Junctions A and B between fragments, Figure 5C shows sequences located at Junction C and Figure 5D shows sequences located at Junctions D and E (SEQ ID NO's: 17, 18, 21, 26, and 28). Figures 5B to 5D also describe the restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), pseudorabies virus (PRV), g50 (gpD), glycoprotein 63 (gp63), early promoter 1 (EP1), late promoter 1 (LP1) (SEQ ID NO: 46), late promoter 2 (LP2), lactose operon Z gene (lacZ), and Escherichia coli (*E. coli*).

Figure 6

Western blot of lysates from recombinant SPV infected cells with anti-serum to PRV. Lanes (A) uninfected Vero cell lysate, (B) S-PRV-000 (pseudorabies virus S62/26) infected cell lysate, (C) pre-stained molecular weight markers, (D) uninfected EMSK cell lysate, (E) S-

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SPV-000 infected cell lysate, (F) S-SPV-003 infected cell lysate, (G) S-SPV-008 infected cell lysate. Cell lysates were prepared as described in the PREPARATION OF INFECTED CELL LYSATES. Approximately 1/5 of the total lysate sample was loaded in each lane.

## Figure 7

DNA sequence of NDV Hemagglutinin-Neuraminidase gene (HN) (SEQ ID NO: 29). The sequence of 1907 base pairs of the NDV HN cDNA clone are shown. The translational start and stop of the HN gene is indicated by the amino acid translation below the DNA sequence.

Figures 8A, 8B, 8C, and 8D

show a detailed description of the DNA insertion in Homology Vector 538-46.26. Figure 5A contains a diagram showing the orientation of DNA fragments assembled in plasmid 538-46.26 and table indicating the origin of each fragment. Figure 8B shows the sequences located at Junctions A and B between fragments; Figure 8C shows the sequences located at Junctions C and D, Figure 8D shows the sequences located at Junction E (SEQ ID NO's: 31, 32, 34, 37, and 40). The restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction in Figures 8B and 8D. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The



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following abbreviations are used, swinepox virus (SPV), Newcastle Disease virus (NDV), hemagglutinin-neuraminidase (HN), early promoter 1 (EP1), late promoter 1 (LP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and Escherichia coli (E. coli).

Figures 9A, 9B, and 9C

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show a detailed description of Swinepox Virus S-SPV-010 and the DNA insertion in Homology Vector 561-36.26. Figure 9A contains a diagram showing the orientation of DNA fragments assembled in plasmid 561-36.26 and a table indicating the origin of each fragment. Figure 9B shows the sequences located at Junctions A and B between fragments and Figure 9C show the sequences located at junction C and D (SEQ ID. NO: 47, 48, 49,50). The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 9B and 9C. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), thymidine kinase (TK), pox synthetic late promoter 1 (LP1), base pairs (BP).

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Figures 10A, 10B, 10C, and 10D

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show a detailed description of Swinepox Virus S-SPV-011 and the DNA insertion in Homology Vector 570-91.21. Figure 10A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.21 and a table indicating the origin of each fragment. Figure 10B show the sequences located at Junctions A and B between fragments; Figure 10C shows the sequences located at Junction C, and Figure 10D shows the sequences located at Junctions 10D and 10E (SEQ ID NO: 51, 52, 53, 54, 55). The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 10B to 10D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 2 (EP2) (SEQ ID NO: 45), gIII (gpC), base pairs (BP).

Figures 11, 11B, 11C and 11D

show a detailed description of Swinepox Virus S-SPV-012 and the DNA insertion in Homology Vector 570-91.41. Figure 11A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.41 and a table indicating the origin of each fragment. Figure 11B shows the sequences located at Junctions A and B between fragments, Figure 11C shows the sequences located at Junction C, and Figure 11D shows the sequence located at Junctions D and E. (SEQ ID NO: 56,

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57, 58, 59, 60). The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 11B to 11D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2 (EP1LP2) (SEQ ID NO: 43), gIII (gpC), base pairs (BP).

Figures 12, 12B, 12C and 12D

show a detailed description of Swinepox Virus S-PRV-013 and the DNA insertion in Homology Vector 570-91.64. Figure 12A contains a diagram showing the orientation of DNA fragments assembled in plasmid 570-91.64 and a table indicating the origin of each fragment. Figure 12B shows the sequences located at Junctions A and B between fragments, Figure 12C shows the sequences located at Junction C, and Figure 12D shows the sequences located at Junctions D and E (SEQ ID NO: 61, 62, 63, 64, 65). The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 12B to 12D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate

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the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2) (SEQ ID NO: 44), gIII (gpC) base pairs (BP).

Figures 13A, 13B, 13C and 13D

show a detailed description of Swinepox Virus S-PRV-014 and the DNA insertion in Homology Vector 599-65.25. Figure 13A contains a diagram showing the orientation of DNA fragments assembled in plasmid 599-65.25 and a table indicating the origin of each fragment. Figure 13B shows sequences located at Junctions A and B between the fragments, Figure 13C shows sequences located at Junction C, and Figure 13D shows sequences located at Junctions D and E. (SEQ ID NO: 66, 67, 68, 69, 70). The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 13B to 13D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), infectious laryngotracheitis virus (ILT), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2 (EP1LP2), glycoprotein G (gpG), polymerase chain reaction (PCR), base pairs (BP).

Figures 14A, 14B, 14C, and 14D

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show a detailed description of Swinepox Virus S-SPV-016 and the DNA insertion in Homology Vector 624-20.1C. Figure 14A contains a diagram showing the orientation of DNA fragments assembled in plasmid 624-20.1C and a table indicating the origin of each fragment. Figure 14B shows the sequences located at Junctions A and B between fragments; Figure 14C shows the sequences located at Junction C, and Figure 14D shows the sequences at Junctions D and E. (SEQ ID NO: 71, 72, 73, 74, 75). The restriction sites are used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 14B to 14D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), infectious laryngotracheitis virus (ILT), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein I (gpI), polymerase chain reaction (PCR), base pairs (BP).

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Figures 15A, 15B, 15C and 15D

show a detailed description of Swinepox Virus S-SPV-017 and the DNA insertion in Homology Vector 614-83.18. Figure 15A contains a diagram showing the orientation of DNA fragments assembled in plasmid 614-83.18 and a table showing the origin of each fragment. Figure 15B shows the sequences located at Junctions A and B between fragments, Figure 15C shows the sequences at Junction C, and Figure 15D shows the sequences located

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at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 15B to 15D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), infectious bovine rhinotracheitis virus (IBR), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein G (gpG), polymerase chain reaction (PCR), base pairs (BP).

Figure 16

Western blot of lysates from recombinant SPV infected cells with polyclonal goat anti-PRV gIII (gpC). Lanes (A) S-PRV-002 (U.S. Patent No. 4,877,737, issued October 31, 1989) infected cell lysate, (B) molecular weight markers, (C) mock-infected EMSK cell lysate, (D) S-SPV-003 infected cell lysate, (E) S-SPV-008 infected cell lysate, (F) S-SPV-011 infected cell lysate, (G) S-SPV-012 infected cell lysate, (H) S-SPV-013 infected cell lysate. Cell lysates are prepared as described in the PREPARATION OF INFECTED CELL LYSATES. Approximately 1/5 of the total lysates sample is loaded in each lane.

Figure 17

Map showing the 3,628 base pair BglIII to HindIII swinepox virus DNA fragment inserted into homology vector 515-85.1.

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Two open reading frames, O2L and O1L, are shown with the number of amino acids coding in each open reading frame. The homology vector 738-94.5 contains a deletion of SPV DNA from nucleotides 1381 to 2133 (SEQ ID NO. 113). The lacZ gene has  
5 been inserted into this region and is expressed from the O1L promoter. Positions of restriction sites AccI, BglII, and HindIII are shown.

Figures 18A, 18B, 18C and 18D

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show a detailed description of Swinepox Virus S-SPV-034 and the DNA insertion in Homology Vector 723-59A9.22. Figure 18A contains a diagram showing the orientation of DNA fragments assembled in plasmid 723-59A9.22 and  
15 a table indicating the origin of each fragment. Figure 18B shows the sequences located at Junctions A and B between fragments, Figure 18C shows the sequences located at Junction C, and Figure 18D shows the sequences located at Junctions D and E. The  
20 restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 18B to 18D. The location of several gene coding regions and regulatory elements is also given.  
25 The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), equine  
30 influenza virus (EIV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), neuraminidase (NA), Prague (PR), polymerase chain reaction (PCR), base pairs (BP).

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## Figures 19A, 19B, 19C and 19D

show a detailed description of Swinepox Virus S-SPV-015 and the DNA insertion in Homology Vector 727-54.60. Figure 19A contains a diagram showing the orientation of DNA fragments assembled in plasmid 727-54.60 and a table indicating the origin of each fragment. Figure 19B shows the sequences located at Junctions A and B between fragments, Figure 19C shows the sequences located at Junction C, and Figure 19D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 19B to 19D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein B (gB), base pairs (BP).

## Figures 20A, 20B, 20C, and 20D

show a detailed description of Swinepox Virus S-SPV-031 and the DNA insertion in Homology Vector 727-67.18. Figure 20A contains a diagram showing the orientation of DNA fragments assembled in plasmid 727-67.18 and a table indicating the origin of each fragment. Figure 20B shows the sequences located at Junctions A and B between fragments, Figure 20C shows the sequences



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located at Junction C, and Figure 20D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 20B to 20D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2 (EP1LP2), antigen (Ag), base pairs (BP).

Figures 21A, 21B, 21C and 21D

show a detailed description of Swinepox Virus S-SPV-033 and the DNA insertion in Homology Vector 732-18.4. Figure 21A contains a diagram showing the orientation of DNA fragments assembled in plasmid 732-18.4 and a table indicating the origin of each fragment. Figure 21B shows the sequences located at Junctions A and B between fragments, Figure 21C shows the sequences located at Junction C, and Figure 21D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 21B to 21D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites

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which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), equine influenza virus (EIV), neuraminidase (NA), Alaska (AK), polymerase chain reaction (PCR), base pairs (BP).

Figures 22A, 22B and 22C

show a detailed description of Swinepox Virus S-SPV-036 and the DNA insertion in Homology Vector 741-80.3. Figure 22A contains a diagram showing the orientation of DNA fragments assembled in plasmid 741-80.3 and a table indicating the origin of each fragment. Figure 22B shows the sequences located at Junctions A, B, and C between fragments and Figure 22C shows the sequences located at Junctions D, E and F. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 22B and 22C. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), human cytomegalovirus immediate early (HCMV IE), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), polyadenylation site (poly A), base pairs (BP).

Figures 23A, 23B, 23C and 23D

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show a detailed description of Swinepox Virus S-SPV-035 and the DNA insertion in Homology Vector 741-84.14. Figure 23A contains a diagram showing the orientation of DNA fragments assembled in plasmid 741-84.14 and a table indicating the origin of each fragment. Figure 23B shows the sequences located at Junctions A and B between fragments, Figure 23C shows the sequences located at Junction C, and Figure 23D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 23B to 23D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), pseudorabies virus (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), interleukin-2 (IL-2), glycoprotein X (gX) polymerase chain reaction (PCR), sequence (seq), base pairs (BP).

Figures 24A, 24B, 24C and 24D

show a detailed description of Swinepox Virus S-SPV-038 and the DNA insertion in Homology Vector 744-34. Figure 24A contains a diagram showing the orientation of DNA fragments assembled in plasmid 744-34 and a table indicating the origin of each fragment. Figure 24B shows the sequences located at Junction A and B between fragments, Figure 24C shows the sequences located at Junction C, and Figure 24D shows the

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sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 24B and 24D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), equine herpesvirus type 1 (EHV-1), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein B (gB), polymerase chain reaction (PCR), base pairs (BP).

Figures 25A, 25B, 25C, and 25D

show a detailed description of Swinepox Virus S-SPV-039 and the DNA insertion in Homology Vector 744-38. Figure 25A contains a diagram showing the orientation of DNA fragments assembled in plasmid 744-38 and a table indicating the origin of each fragment. Figure 25B shows the sequences located at Junction A and B between fragments. Figure 25C shows the sequences located at Junction C and Figure 25D shows the sequences located at Junctions D and E. The restriction sites used to generate each fragment as well as synthetic linker sequences which are used to join the fragments are described for each junction in Figures 25B to 25D. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parentheses, (), refer to amino acids, and restriction sites in brackets, [], indicate the remnants of sites

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which are destroyed during construction. The following abbreviations are used: swinepox virus (SPV), equine herpesvirus type 1 (EHV-1), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), glycoprotein D (gD), polymerase chain reaction (PCR), base pairs (BP).

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Detailed Description Of The Invention

The present invention provides a recombinant swinepox virus (SPV) capable of replication in an animal into which the recombinant swinepox virus is introduced which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in the animal into which the recombinant swinepox virus is introduced, the foreign DNA being inserted into the swinepox viral DNA at an insertion site which is not essential for replication of the swinepox virus and being under the control of a promoter.

For purposes of this invention, "a recombinant swinepox virus capable of replication" is a live swinepox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant swinepox virus deleted.

For purposes of this invention, "an insertion site which is not essential for replication of the swinepox virus" is a location in the swinepox viral genome where a sequence of DNA is not necessary for viral replication, for example, complex protein binding sequences, sequences which code for reverse transcriptase or an essential glycoprotein, DNA sequences necessary for packaging, etc.

For purposes of this invention, a "promoter" is a specific DNA sequence on the DNA molecule to which the foreign RNA polymerase attaches and at which transcription of the foreign RNA is initiated.

The invention further provides foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in

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the animal. Preferably, this antigenic polypeptide is a linear polymer of more than 10 amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

- 5 The invention further provides an insertion site present within the larger HindIII to BglII subfragment of the HindIII M fragment of swinepox viral DNA. Preferably, the insertion site is within an open reading frame contained in the HindIII to BglII subfragment. Preferably, the insertion  
10 site is the AccI restriction endonuclease site located in the HindIII to BglII subfragment.

- The invention further provides an insertion site within an open reading frame encoding swinepox thymidine kinase.  
15 Preferably, the insertion site is the NdeI restriction endonuclease site located within the swinepox virus thymidine kinase gene.

- For purposes of this invention, an "open reading frame" is  
20 a segment of DNA which contains codons that can be transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

- 25 The invention further provides a recombinant swinepox virus capable of replication which contains a foreign DNA encoding a polypeptide which is a detectable marker. Preferably the detectable marker is the polypeptide E. coli  $\beta$ -galactosidase. Preferably, the insertion site for the  
30 foreign DNA encoding E. coli  $\beta$ -galactosidase is the AccI restriction endonuclease site located within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox virus is designated S-SPV-003 (ATCC Accession No. VR 2335). The S-SPV-003 swinepox virus has  
35 been deposited pursuant to the Budapest Treaty on the

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International Deposit of Microorganisms for the Purposes of  
Patent Procedure with the Patent Culture Depository of the  
American Type Culture Collection, 12301 Parklawn Drive,  
Rockville, Maryland 20852 U.S.A. under ATCC Accession No.  
5 VR 2335.

For purposes of this invention, a "polypeptide which is a  
detectable marker" includes the bimer, trimer and tetramer  
form of the polypeptide. E. coli  $\beta$ -galactosidase is a  
10 tetramer composed of four polypeptides or monomer sub-units.

The invention further provides a recombinant swinepox virus  
capable of replication which contains foreign DNA encoding  
an antigenic polypeptide which is or is from pseudorabies  
15 virus (PRV) g50 (gpD), pseudorabies virus (PRV) II (gpB),  
Pseudorabies virus (PRV) gIII (gpC), pseudorabies virus  
(PRV) glycoprotein H, pseudorabies virus (PRV) glycoprotein  
E, Transmissible gastroenteritis (TGE) glycoprotein 195,  
Transmissible gastroenteritis (TGE) matrix protein, swine  
20 rotavirus glycoprotein 38, swine parvovirus capsid protein,  
Serpulina hyodysenteriae protective antigen, Bovine Viral  
Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus  
(NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin  
or swine flu neuraminidase. Preferably, the antigenic  
25 polypeptide is Pseudorabies Virus (PRV) g50 (gpD).  
Preferably, the antigenic protein is Newcastle Disease Virus  
(NDV) hemagglutinin-neuraminidase.

The invention further provides a recombinant swinepox virus  
30 capable of replication which contains foreign DNA encoding  
an antigenic polypeptide which is or is from Serpulina  
hyodysenteriae, Foot and Mouth Disease Virus, Hog Cholera  
Virus, Swine Influenza Virus, African Swine Fever Virus or  
Mycoplasma hyopneumoniae.

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The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) g50 (gpD). This recombinant swinepox virus can be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral genome for insertion of the foreign DNA encoding PRV g50 (gpD) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox virus is designated S-SPV-008 (ATCC Accession No. VR 2339). The S-SPV-008 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2339.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) gIII (gpC). This recombinant swinepox virus can also be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral DNA for insertion of the foreign DNA encoding PRV C gene and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox virus is designated S-SPV-011, S-SPV-012, or S-SPV-013. The swinepox virus designated S-SPV-013 has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR \_\_\_\_.

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The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) gII (gpB). This recombinant swinepox virus can also be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral DNA for insertion of the foreign DNA encoding PRV gII (gpB) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox virus is designated S-SPV-015 (ATCC Accession No. VR \_\_\_\_\_). The S-SPV-015 swinepox virus has been deposited on July 22, 1994 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR \_\_\_\_\_.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) g50 (gpD) and foreign DNA encoding pseudorabies virus (PRV) gIII (gpC). This recombinant swinepox virus can also be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral DNA for insertion of the foreign DNA encoding PRV g50 (gpD), PRV gIII (gpC) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) g50 (gpD) and foreign DNA encoding pseudorabies virus (PRV) gII (gpB). This recombinant swinepox virus can also be further engineered to

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contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral genome for insertion of foreign DNA encoding PRV g50 (gpD), PRV gII (gpB) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) gIII (gpC) and foreign DNA encoding pseudorabies virus (PRV) gII (gpB). This recombinant swinepox virus can also be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase. A preferred site within the swinepox viral genome for insertion of foreign DNA encoding PRV gIII (gpC), PRV gII (gpB) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding pseudorabies virus (PRV) g50 (gpD), foreign DNA encoding pseudorabies virus (PRV) gIII (gpC), and foreign DNA encoding pseudorabies virus (PRV) gII (gpB). This recombinant swinepox virus can also be further engineered to contain foreign DNA encoding a detectable marker, such as *E. coli* B-galactosidase.

A preferred site within the swinepox viral genome for insertion of foreign DNA encoding PRV g50 (gpD), PRV gIII (gpC), PRV gII (gpB) and *E. coli* B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA.

The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA

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encoding RNA encoding the antigenic polypeptide Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker. Preferably, this recombinant swinepox virus is designated S-SPV-009 (ATCC Accession No. VR 2344). The S-SPV-009 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2344.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis virus glycoprotein E and glycoprotein G. Preferred embodiment of this invention are recombinant swinepox viruses designated S-SPV-017 and S-SPV-019.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis virus and is capable of being expressed in a host infected by the recombinant swinepox virus. Examples of such antigenic polypeptide are infectious laryngotracheitis virus glycoprotein G and glycoprotein I. Preferred embodiment of this invention are recombinant swinepox viruses designated S-SPV-014 and S-SPV-016.

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The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.

For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant tumor from the group consisting of *Plasmodium falciparum*, *Bordetella pertussis*, and malignant tumor.

In one embodiment of the invention, a recombinant swinepox virus contains the foreign DNA sequence encoding hepatitis B virus core protein. Preferably, such virus recombinant virus is designated S-SPV-031.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant swinepox virus and is capable of being expressed in the host infected.

For example, the cytokine can be, but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons,

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granulocyte-macrophage colony stimulating factors, and interleukin receptors.

5 In one embodiment of the invention, a recombinant swinepox virus contains a foreign DNA sequence encoding human interleukin-2. Preferably, such recombinant virus is designated S-SPV-035.

10 The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant  
15 swinepox virus.

The antigenic polypeptide of an equine pathogen can derived from equine influenza virus or equine herpesvirus. Examples of such antigenic polypeptide are equine influenza virus  
20 type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein  
25 D. Preferred embodiments of such recombinant virus are designated S-SPV-033, S-SPV-034, S-SPV-038, and S-SPV-039.

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence  
30 inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus, and is capable of being expressed in a host infected by the recombinant swinepox  
35 virus.

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For example, the antigenic polypeptide of derived from bovine respiratory syncytial virus equine pathogen can derived from equine influenza virus is bovine respiratory syncytial virus attachment protein (BRSV G), bovine  
5 respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

10

Preferred embodiments of a recombinant virus containing a foreign DNA encoding an antigenic polypeptide from a bovine respiratory syncytial virus are designated S-SPV-020, S-SPV-029, and S-SPV-030.

15

And a preferred embodiment of a recombinant virus containing a foreign DNA encoding an antigenic polypeptide from a bovine parainfluenza virus are designated S-SPV-028.

20

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes bovine viral diarrhea virus glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being  
25 expressed in a host infected by the recombinant swinepox virus. Preferred embodiments of such virus are designated S-SPV-032 and S-SPV-040.

30

The present invention further provides a recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease virus and wherein the foreign DNA sequence is capable of being  
35 expressed in a host infected by the recombinant swinepox

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virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and VP2. Preferred embodiments of such virus are designated S-SPV-026 and S-SPV-027.

5

The invention further provides that the inserted foreign DNA sequence is under the control of a promoter. Preferably, the promoter is a swinepox viral promoter. Preferably, the promoter is a synthetic pox viral promoter. For purposes of  
10 this invention, the promoters were generated by methods well known to those of skill in the art, for example, as set forth in the STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS in Materials and Methods. For purposes of  
15 this invention, a synthetic pox promoter includes a synthetic late pox promoter, a synthetic early pox promoter or a synthetic early/late pox promoter.

The invention provides for a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into  
20 the genomic DNA of a swinepox virus. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced, with at one end of  
25 the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus, and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA  
30 located at the other side of the same site on the genomic DNA. Preferably, the RNA encodes a polypeptide.

In one embodiment, the polypeptide is a detectable marker. Preferably, the polypeptide which is a detectable marker is  
35 E. coli  $\beta$ -galactosidase.



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In one embodiment, the polypeptide is antigenic in the animal. Preferably, the antigenic polypeptide is or is from pseudorabies virus (PRV) g50 (gpD), pseudorabies virus (PRV) gII (gpB), Pseudorabies virus (PRV) gIII (gpC), Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, Serpulina hyodysenteriae protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from Serpulina hyodysenteriae, Foot and Mouth Disease Virus, Hog Cholera Virus, Swine Influenza Virus, African Swine Fever Virus or Mycoplasma hyopneumoniae.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from a human pathogen.

For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicell-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant tumor from the group consisting of *Plasmodium falciparum*, *Bordetella pertussis*, and malignant tumor.

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes a cytokine capable of stimulating human immune response.

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For example, the cytokine can be, but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin receptors.

5

In an embodiment of the present invention, the double stranded foreign DNA sequence in the homology vector encodes an antigenic polypeptide derived from an equine pathogen.

10 The antigenic polypeptide of an equine pathogen can derived from equine influenza virus or equine herpesvirus. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type  
15 A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.

20 In an embodiment of the present invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus.

25 For example, the antigenic polypeptide of derived from bovine respiratory syncytial virus equine pathogen can derived from equine influenza virus is bovine respiratory syncytial virus attachment protein (BRSV G), bovine  
30 respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

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In an embodiment of the present invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from infectious bursal disease virus. Examples of such antigenic polypeptide are  
5 infectious bursal disease virus polyprotein and infectious bursal disease virus VP2.

In another embodiment of the present invention, the double-stranded swinepox viral DNA of the homology vectors  
10 described above is homologous to genomic DNA present within the larger HindIII to BglII subfragment of the HindIII M fragment of swinepox virus. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame contained in this HindIII to  
15 BglII subfragment. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the AccI restriction endonuclease site located in this HindIII to BglII subfragment.

20 For purposes of this invention, a "homology vector" is a plasmid constructed to insert foreign DNA in a specific site on the genome of a swinepox virus.

In one embodiment of the invention, the double-stranded  
25 swinepox viral DNA of the homology vectors described above is homologous to genomic DNA present within the open reading frame encoding swinepox thymidine kinase. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the NdeI restriction endonuclease site  
30 located in the open reading frame encoding swinepox thymidine kinase.

The invention further provides a homology vectors described above, the foreign DNA sequence of which is under control of  
35 a promoter located upstream of the foreign DNA sequence.

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The promoter can be an endogenous swinepox viral promoter or an exogenous promoter. The promoter can be a synthetic pox viral promoter or human cytomegalovirus immediate early gene promoter.

5

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox virus of the present invention and a suitable carrier.

10 Suitable carriers for the pseudorabies virus are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

15

For purposes of this invention, an "effective immunizing amount" of the recombinant swinepox virus of the present invention is within the range of  $10^3$  to  $10^9$  PFU/dose.

20 The present invention also provides a method of immunizing an animal, wherein the animal is a human, swine, bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the virus or viruses which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine  
25 parvovirus, Serpulina hyodysenteriae, bovine viral diarrhea, Newcastle disease, swine flu, foot and mouth disease, hog cholera, African swine fever or Mycoplasma hyopneumoniae. For purposes of this invention, the method of immunizing  
30 also includes immunizing the animal against human pathogens, bovine pathogens, equine pathogens, avian pathogens described in the preceding part of this section.

The method comprises administering to the animal an  
35 effective immunizing dose of the vaccine of the present

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invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may be  
5 administered intranasally or orally.

The present invention also provides a method for testing a swine to determine whether the swine has been vaccinated with the vaccine of the present invention, particularly the  
10 embodiment which contains the recombinant swinepox virus S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies virus. This method comprises obtaining from the swine to be tested a sample of a suitable body fluid, detecting in the sample the  
15 presence of antibodies to pseudorabies virus, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies virus are present, detecting in the sample the absence of antibodies to pseudorabies virus  
20 antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies virus but which are not present in a vaccinated swine indicating that the swine was vaccinated and is not infected.

25 The present invention also provides a host cell infected with a recombinant swinepox virus capable of replication. In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero cell. Preferably, the mammalian cell is an ESK-4 cell, PK-15 cell or EMSK  
30 cell.

For purposes of this invention a "host cell" is a cell used to propagate a vector and its insert. Infecting the cells was accomplished by methods well known to those of skill in

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the art, for example, as set forth in INFECTION -  
TRANSFECTION PROCEDURE in Material and Methods.

Methods for constructing, selecting and purifying  
5 recombinant swinepox viruses described above are detailed  
below in Materials and Methods.

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Materials and Methods

PREPARATION OF SWINEPOX VIRUS STOCK SAMPLES. Swinepox virus (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells, ESK-4 cells, PK-15 cells or Vero cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of Iscove's Modified Dulbecco's Medium (IMDM) and RPMI 1640 medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as EMSK negative medium). Prior to infection, the cell monolayers were washed once with EMSK negative medium to remove traces of fetal bovine serum. The SPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete EMSK medium (EMSK negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO<sub>2</sub> until cytopathic effect was complete. The medium and cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10<sup>6</sup> PFU/ml.

PREPARATION OF SPV DNA. For swinepox virus DNA isolation, a confluent monolayer of EMSK cells in a T175 cm<sup>2</sup> flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in 1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na<sub>2</sub>HPO<sub>4</sub>, 0.2g KH<sub>2</sub>PO<sub>4</sub>, 0.8g NaCl and

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0.2g KCl per liter H<sub>2</sub>O) (per T175) and subjected to two successive freeze-thaws (-70° C to 37° C). Upon the last thaw, the cells (on ice) were sonicated two times for 30 seconds each with 45 seconds cooling time in between.

5 Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4° C. SPV virions, present in the supernatant, were then pelleted by centrifugation at 15,000 rpm for 20 minutes at 4° C in a SS34 rotor (Sorvall) and resuspended in

10 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60 minutes in a SW41 rotor (Beckman) at 4° C. The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and

15 sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at 4° C. The SPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and

20 pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4° C. The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces of sucrose and finally resuspended in 10 mM Tris pH 7.5. SPV DNA was then extracted from the purified virions by lysis (4 hours at 60°

25 C) induced by the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and

30 incubation at -20° C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4° C.

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PREPARATION OF INFECTED CELL LYSATES. For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (EMSK, ESK-4, PK-15 or Vero for SPV or VERO for PRV) in a 25 cm<sup>2</sup> flask or a 60 mm petri dish was infected with 100 µl of virus sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 µl of disruption buffer (2% sodium dodecyl sulfate, 2% β-mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C.

WESTERN BLOTTING PROCEDURE. Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemmli (1970). After gel electrophoresis the proteins were transferred and processed according to Sambrook *et al.* (1982). The primary antibody was a swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H<sub>2</sub>O). The secondary antibody was a goat anti-swine alkaline phosphatase conjugate diluted 1:1000 with TSA.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis *et al.* (1982) and Sambrook *et al.* (1989). Except as noted, these were used with minor variation.

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DNA SEQUENCING. Sequencing was performed using the USE Sequenase Kit and <sup>35</sup>S-dATP (NEN). Reactions using both the dGTP mixes and the dITP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar software. Manipulation and comparison of sequences obtained was performed with Superclone™ and Supersee™ programs from Coral Software.

CLONING WITH THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, et al. (1990). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each case are detailed in the descriptions of the construction of homology vectors below.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001 (Kasza SPV strain, 17) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION -

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TRANSFECTION PROCEDURE. The construction of homology vectors used in this procedure is described below

INFECTION - TRANSFECTION PROCEDURE. 6 cm plates of EMSK  
5 cells (about 80% confluent) were infected with S-SPV-001 at  
a multiplicity of infection of 0.01 PFU/cell in EMSK  
negative medium and incubated at 37°C in a humidified 5% CO<sub>2</sub>  
environment for 5 hours. The transfection procedure used is  
essentially that recommended for Lipofectin™ Reagent (BRL).  
10 Briefly, for each 6 cm plate, 15 µg of plasmid DNA was  
diluted up to 100 µl with H<sub>2</sub>O. Separately, 50 micrograms of  
Lipofectin Reagent was diluted to 100 µl with H<sub>2</sub>O. The 100  
µl of diluted Lipofectin Reagent was then added dropwise to  
the diluted plasmid DNA contained in a polystyrene 5 ml snap  
15 cap tube and mixed gently. The mixture was then incubated  
for 15-20 minutes at room temperature. During this time, the  
virus inoculum was removed from the 6 cm plates and the cell  
monolayers washed once with EMSK negative medium. Three ml  
of EMSK negative medium was then added to the plasmid  
20 DNA/lipofectin mixture and the contents pipetted onto the  
cell monolayer. The cells were incubated overnight (about 16  
hours) at 37°C in a humidified 5% CO<sub>2</sub> environment. The next  
day the 3 ml of EMSK negative medium was removed and  
replaced with 5 ml EMSK complete medium. The cells were  
25 incubated at 37°C in 5% CO<sub>2</sub> for 3-7 days until cytopathic  
effect from the virus was 80-100%. Virus was harvested as  
described above for the preparation of virus stocks. This  
stock was referred to as a transfection stock and was  
subsequently screened for recombinant virus by the BLUOGAL  
30 SCREEN FOR RECOMBINANT SWINEPOX VIRUS OR CPRG SCREEN FOR  
RECOMBINANT SWINEPOX VIRUS.

SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE  
(BLUOGAL AND CPRG ASSAYS). When the E. coli β-galactosidase  
35 (lacZ) marker gene was incorporated into a recombinant virus

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the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical Bluogal™ (Bethesda Research Labs) was incorporated (200 µg/ml) into the agarose overlay during the plaque assay, and 5 plaques expressing active β-galactosidase turned blue. The blue plaques were then picked onto fresh cells (EMSK) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 µg/ml) into the agarose overlay during the plaque assay, and 10 plaques expressing active β-galactosidase turned red. The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases viruses were typically purified with three rounds of plaque purification.

15

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox viruses, monolayers of EMSK cells were infected with recombinant SPV, 20 overlaid with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. Fixation of the cells results in 25 cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate dilution with PBS and incubated on the cell monolayer for 2 hours at room temperature. To 30 detect PRV g50 (gpD) expression from S-SPV-008, swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) was used (diluted 1:100). To detect NDV HN expression from S-SPV-009, a rabbit antiserum specific for the HN protein (rabbit anti-NDV#2) was used (diluted 1:1000). Unbound 35 antibody was then removed by washing the cells three times

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with PBS at room temperature. The secondary antibody, either a goat anti-swine (PRV g50 (gpD); S-SPV-008) or goat anti-rabbit (NDV HN; S-SPV-009), horseradish peroxidase conjugate was diluted 1:250 with PBS and incubated with the cells for 5 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The cells were then incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100  $\mu$ g/ml 4-chloro-1-naphthol, 0.003%  $H_2O_2$  in PBS). 10 Plaques expressing the correct antigen stain black.

PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS. Viral glycoproteins are purified using antibody affinity columns. To produce monoclonal 15 antibodies, 8 to 10 week old BALB/c female mice are vaccinated intraperitoneally seven times at two to four week intervals with  $10^7$  PFU of S-SPV-009, -014, -016, -017, -018, or -019. Three weeks after the last vaccination, mice are injected intraperitoneally with 40 mg of the corresponding 20 viral glycoprotein. Spleens are removed from the mice three days after the last antigen dose.

Splenocytes are fused with mouse NS1/Ag4 plasmacytoma cells by the procedure modified from Oi and Herzenberg, (41). 25 Splenocytes and plasmacytoma cells are pelleted together by centrifugation at 300 x g for 10 minutes. One ml of a 50% solution of polyethylene glycol (m.w. 1300-1600) is added to the cell pellet with stirring over one minute. Dulbecco's modified Eagles's medium (5ml) is added to the cells over 30 three minutes. Cells are pelleted by centrifugation at 300 x g for 10 minutes and resuspended in medium with 10% fetal bovine serum and containing 100 mM hypoxanthine, 0.4 mM aminopterin and 16 mM thymidine (HAT). Cells (100 ml) are added to the wells of eight to ten 96-well tissue culture 35 plates containing 100 ml of normal spleen feeder layer cells

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and incubated at 37°C. Cells are fed with fresh HAT medium every three to four days.

Hybridoma culture supernatants are tested by the ELISA ASSAY  
5 in 96-well microtiter plates coated with 100 ng of viral glycoprotein. Supernatants from reactive hybridomas are further analyzed by black-plaque assay and by Western Blot. Selected hybridomas are cloned twice by limiting dilution. Ascetic fluid is produced by intraperitoneal injection of 5  
10  $\times 10^6$  hybridoma cells into pristane-treated BALB/c mice.

Cell lysates from S-SPV-009, -014, -016, -017, -018, or -019 are obtained as described in PREPARATION OF INFECTED CELL  
15 LYSATES. The glycoprotein-containing cell lysates (100 mls) are passed through a 2-ml agarose affinity resin to which 20 mg of glycoprotein monoclonal antibody has been immobilized according to manufacturer's instructions (AFC Medium, New Brunswick Scientific, Edison, N.J.). The column is washed  
20 with 100 ml of 0.1% Nonidet P-40 in phosphate-buffered saline (PBS) to remove nonspecifically bound material. Bound glycoprotein is eluted with 100 mM carbonate buffer, pH 10.6 (40). Pre- and posteluted fractions are monitored for purity by reactivity to the SPV monoclonal antibodies in  
25 an ELISA system.

ELISA ASSAY. A standard enzyme-linked immunosorbent assay (ELISA) protocol is used to determine the immune status of cattle following vaccination and challenge.

30 A glycoprotein antigen solution (100 ml at ng/ml in PBS) is allowed to absorb to the wells of microtiter dishes for 18 hours at 4°C. The coated wells are rinsed one time with PBS. Wells are blocked by adding 250 ml of PBS containing 1% BSA (Sigma) and incubating 1 hour at 37°C. The blocked  
35 wells are rinsed one time with PBS containing 0.02% Tween

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20. 50 ml of test serum (previously diluted 1:2 in PBS containing 1% BSA) are added to the wells and incubated 1 hour at 37°C. The antiserum is removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 50 ml of  
5 a solution containing anti-bovine IgG coupled to horseradish peroxidase (diluted 1:500 in PBS containing 1% BSA, Kirkegaard and Perry Laboratories, Inc.) is added to visualize the wells containing antibody against the specific antigen. The solution is incubated 1 hour at 37°C, then  
10 removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 100 ml of substrate solution (ATBS, Kirkegaard and Perry Laboratories, Inc.) are added to each well and color is allowed to develop for 15 minutes. The reaction is terminated by addition of 0.1M oxalic acid. The  
15 color is read at absorbance 410nm on an automatic plate reader.

STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS. For recombinant swinepox vectors synthetic pox  
20 promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. We chose to design three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the vaccinia virus (1, 7 and 8). Each cassette was designed to contain  
25 the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either *EcoRI* or *BamHI* sites. A set of  
30 translational stop codons in all three reading frames and an early transcriptional termination signal (9) were also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors  
35 (see Figures 4, 5 and 8).

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VACCINATION STUDIES IN SWINE USING RECOMBINANT SWINEPOX  
VIRUS CONTAINING PSEUDORABIES VIRUS GLYCOPROTEIN GENES:

- Young weaned pigs from pseudorabies-free herd are used to test the efficacy of the recombinant swinepox virus
- 5 containing one or more of the pseudorabies virus glycoprotein genes (SPV/PRV). The piglets are inoculated intradermally or orally about  $10^3$  to  $10^7$  plaque forming units (PFU) of the recombinant SPV/PRV viruses.
- 10 Immunity is determined by measuring PRV serum antibody levels and by challenging the vaccinated pigs with virulent strain of pseudorabies virus. Three to four weeks post-vaccination, both vaccinated and non-vaccinated groups of pigs are challenged with virulent strain of pseudorabies
- 15 virus (VDL4892). Post challenge, the pigs are observed daily for 14 days for clinical signs of pseudorabies.

Serum samples are obtained at the time of vaccination, challenge, and at weekly intervals for two to three weeks

20 post-vaccination and assayed for serum neutralizing antibody.

HOMOLOGY VECTOR 515-85.1. The plasmid 515-85.1 was constructed for the purpose of inserting foreign DNA into

25 SPV. It contains a unique *AccI* restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the *AccI* site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign

30 DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in figure 4. It may be constructed utilizing standard recombinant DNA techniques (22 and 29), by joining two restriction fragments from the following sources. The first fragment is an approximately

35 2972 base pair *HindIII* to *BamHI* restriction fragment of



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pSP64 (Promega). The second fragment is an approximately 3628 base pair *Hind*III to *Bgl*III restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

5   HOMOLOGY VECTOR 520-17.5. The plasmid 520-17.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene flanked by SPV DNA. Upstream of the marker gene is an approximately 2149 base pair fragment of SPV DNA.  
10 Downstream of the marker gene is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the marker gene will result. Note that the  $\beta$ -galactosidase  
15 (*lacZ*) marker gene is under the control of a synthetic early/late pox promoter. A detailed description of the plasmid is given in figure 4. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources  
20 with the synthetic DNA sequences indicated in figure 4. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III  
25 restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1484 base pair *Acc*I to *Bgl*III restriction sub-fragment of the SPV *Hind*III fragment M (23).

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HOMOLOGY VECTOR 538-46.16. The plasmid 538-46.16 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*)  
35 marker gene and the PRV g50 (*gpD*) gene flanked by SPV DNA.

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Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the g50 (*gpD*) gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in figure 5. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 5. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3006 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1571 base pair *Eco*RI to *Stu*I restriction sub-fragment of the PRV *Bam*HI fragment 7 (21). Note that the *Eco*RI site was introduced in to this fragment by PCR cloning. In this procedure the primers described below were used along with a template consisting of a PRV *Bam*HI #7 fragment subcloned into pSP64. The first primer 87.03 (5'- CGCGAATTCGCTCG CAGCGCTATTGGC-3') (SEQ ID NO:41) sits down on the PRV g50 (*gpD*) sequence (26) at approximately amino acid 3 priming toward the 3' end of the gene. The second primer 87.06 (5'- GTAGGAGTGGCTGCTGAAG-3') (SEQ ID NO:42) sits down on the opposite strand at approximately amino acid 174 priming toward the 5' end of the gene. The PCR product may be digested with *Eco*RI and *Sal*I to produce an approximately 509 base pair fragment. The approximately 1049 base pair *Sal*I

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to *StuI* sub-fragment of PRV *BamHI* #7 may then be ligated to the approximately 509 base pair *EcoRI* to *SalI* fragment to generate the approximately 1558 base pair *EcoRI* to *StuI* fragment 3. Fragment 4 is an approximately 1484 base pair  
5 *AccI* to *BglIII* restriction sub-fragment of the SPV *HindIII* fragment M (23).

HOMOLOGY VECTOR 570-91.21. The plasmid 570-91.21 was constructed for the purpose of inserting foreign DNA into  
10 SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*) marker gene and the PRV *gIII* (*gpC*) gene flanked by SPV DNA. Upstream of the foreign DNA genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign  
15 genes is an approximately 2149 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1),  
20 and the *gIII* (*gpC*) gene is under the control of a synthetic early pox promoter (EP2). A detailed description of the plasmid is given in figure 10. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources  
25 with the synthetic DNA sequences indicated in figure 10. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII*  
30 restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *NcoI* to *NcoI* fragment of plasmid 251-41.A, a subfragment of PRV *BamHI* #2 and #9. *EcoRI* linkers have  
35 replaced the *NcoI* and *NcoI* sites at the ends of this

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fragment. Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* fragment M (23). The *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

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HOMOLOGY VECTOR 570-91.41. The plasmid 570-91.41 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*) marker gene and the PRV *gIII* (*gpC*) gene flanked by SPV DNA.  
10 Upstream of the foreign DNA genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a  
15 virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the *gIII* (*gpC*) gene is under the control of a synthetic early late pox promoter (EP1LP2). A detailed description of  
20 the plasmid is given in figure 11. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 11. The plasmid vector is derived from an approximately 2972  
25 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of  
30 plasmid pJF751 (11). Fragment 3 is an approximately 2378 base pair *NcoI* to *NcoI* fragment of plasmid 251-41.A, a subfragment of PRV *BamHI* #2 and #9. *EcoRI* linkers have replaced the *NcoI* and *NcoI* sites at the ends of this fragment. Fragment 4 is an approximately 2149 base pair  
35 *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII*

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fragment M (23). The *AccI* sites in fragments 1 and 4 have been converted to *PstI* sites using synthetic DNA linkers.

HOMOLOGY VECTOR 570-91.64. The plasmid 570-91.64 was  
5 constructed for the purpose of inserting foreign DNA into  
SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*)  
marker gene and the PRV *gIII* (*gpC*) gene flanked by SPV DNA.  
Upstream of the foreign DNA genes is an approximately 1484  
base pair fragment of SPV DNA. Downstream of the foreign  
10 genes is an approximately 2149 base pair fragment of SPV  
DNA. When this plasmid is used according to the HOMOLOGOUS  
RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a  
virus containing DNA coding for the foreign genes will  
result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is  
15 under the control of a synthetic late pox promoter (LP1),  
and the *gIII* (*gpC*) gene is under the control of a synthetic  
late early pox promoter (LP2EP2). A detailed description of  
the plasmid is given in figure 12. It may be constructed  
utilizing standard recombinant DNA techniques (22 and 30),  
20 by joining restriction fragments from the following sources  
with the synthetic DNA sequences indicated in figure 12.  
The plasmid vector is derived from an approximately 2972  
base pair *HindIII* to *BamHI* restriction fragment of pSP64  
(Promega). Fragment 1 is an approximately 1484 base pair  
25 *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII*  
restriction fragment M (23). Fragment 2 is an approximately  
3002 base pair *BamHI* to *PvuII* restriction fragment of  
plasmid pJF751 (11). Fragment 3 is an approximately 2378  
base pair *NcoI* to *NcoI* fragment of plasmid 251-41.A, a  
30 subfragment of PRV *BamHI* #2 and #9. *EcoRI* linkers have  
replaced the *NcoI* and *NcoI* sites at the ends of this  
fragment. Fragment 4 is an approximately 2149 base pair  
*AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII*  
fragment M (23). The *AccI* sites in fragments 1 and 4 have  
35 been converted to *PstI* sites using synthetic DNA linkers.

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HOMOLOGY VECTOR 538-46.26. The plasmid 538-46.26 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E.coli  $\beta$ -galactosidase (lacZ) marker gene and the Newcastle Disease Virus (NDV) hemagglutinin-Neuraminidase (HN) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the HN gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in figure 8. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 8. The plasmid vector is derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2149 base pair HindIII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1810 base pair AvaII to NaeI restriction fragment of a NDV HN cDNA clone. The sequence of the HN cDNA clone is given in figure 7. The cDNA clone was generated from the B1 strain of NDV using standard cDNA cloning techniques (14). Fragment 3 is an approximately 3006 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 1484 base pair AccI to BglII restriction sub-fragment of the SPV HindIII fragment M (23).

HOMOLOGY VECTOR 599-65.25. The plasmid 599-65.25 was constructed for the purpose of inserting foreign DNA into

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SPV. It incorporates an *E. coli* B-galactosidase (*lacZ*) marker gene and the *ILT gpG* gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the *B-galactosidase* (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the *ILT gpG* gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in figure 13. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 13. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 1073 base pair *EcoRI* to *MboI* fragment. Note that the *EcoRI* site was introduced by PCR cloning. In this procedure, the primers described below were used with a template consisting of a 2.6 kb *Sst I* to *Asp718I* subfragment of a 5.1 kb *Asp718I* fragment of *ILT* virus genome. The first primer 91.13 (5'-CCGAATTCCGGCTTCAGTAACATAGGATCG -3') (SEQ ID NO: 81) sits down on the *ILT gpG* sequence at amino acid 2. It adds an additional asparagine residue between amino acids 1 and 2 and also introduces an *EcoRI* restriction site. The second primer 91.14 (5'-GTACCCATACTGGTCGTGGC-3') (SEQ ID NO: 82) sits down on the opposite strand at approximately amino acid 196 priming toward the 5' end of the gene. The PCR product is digested with *EcoRI* and *BamHI* to produce an approximately 454 base pair fragment. The approximately 485 base pair

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MboI sub-fragment of ILT Asp718I (5.1 kb) fragment is ligated to the approximately 454 base pair *EcoRI* to *BamHI* fragment to generate fragment 2 from *EcoRI* to *MboI* which is approximately 939 base pairs (293 amino acids) in length.

5 Fragment 3 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites of fragments 1 and 4 have been converted to *PstI* sites using synthetic

10 DNA linkers.

HOMOLOGY VECTOR 624-20.1C. The plasmid 624-20.1C was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*)

15 marker gene and the ILT *gpI* gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS

20 RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the *B*-galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the ILT *gpI* gene is under the control of a synthetic

25 late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in figure 14. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 14.

30 The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl* II to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately

35 1090 base pair fragment with *EcoRI* and *BamHI* restriction



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sites at the ends synthesized by PCR cloning and containing the entire amino acid coding sequence of the ILT gpI gene. The ILT gpI gene was synthesized in two separate PCR reactions. In this procedure, the primers described below

5 were used with a template consisting the 8.0 kb ILT Asp 718I fragment. The first primer 103.6 (5'-CCGGAATTCGCTACTT GGA

10 GGA

15 GGA

20 GGA

25 GGA

30 GGA

35 GGA

ACTCTGG-3') (SEQ ID NO 83) sits down on the ILT gpI sequence at amino acid number 2 and introduces an *EcoRI* site at the 5' end of the ILT gpI gene. The second primer 103.3 (5'-CATTGTCCCGAGACGGACAG-3') (SEQ ID NO. 84) sits down on the ILT gpI sequence at approximately amino acid 269 on the opposite strand to primer 103.6 and primes toward the 5' end of the gene. The PCR product was digested with *EcoRI* and *BglI* (*BglI* is located approximately at amino acid 209 which is 179 base pairs 5' to primer 2) to yield a fragment 625 base pairs in length corresponding to the 5' end of the ILT gpI gene. The third primer 103.4 (5'-CGCGATCCAACTATCGGTG-3') (SEQ ID NO. 85) sits down on the ILT gpI gene at approximately amino acid 153 priming toward the 3' end of the gene. The fourth primer 103.5 (5'-GCGGATCCACATTCAG ACTTAATCAC-3') (SEQ ID NO. 86) sits down at the 3' end of the ILT gpI gene 14 base pairs beyond the UGA stop codon, introducing a *BamHI* restriction site and priming toward the 5' end of the gene. The PCR product is digested with *BglI* (at amino acid 209) and *BamHI* to yield a fragment 476 base pairs in length corresponding to the 3' end of the ILT gpI gene. Fragment 2 consists of the products of the two PCR reactions ligated together to yield an ILT gpI gene which is a *EcoRI* to *BamHI* fragment approximately 1101 base pairs (361 amino acids) in length. Fragment 3 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

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HOMOLOGY VECTOR 614-83.18. The plasmid 614-83.18 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*) marker gene and the IBR gpG gene flanked by SPV DNA.

5 Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a

10 virus containing DNA coding for the foreign genes will result. Note that the *B*-galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the IBR gG gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of

15 the plasmid is given in figure 15. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 15. The plasmid vector is derived from an approximately 2972

20 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 1085 base pair fragment synthesized by PCR cloning with

25 *Eco*RI and *Bam*HI restriction sites at the ends and containing the amino acid coding sequence from amino acids 2 to 362 of the IBR gpG gene. In the PCR cloning procedure, the primers described below were used with a template consisting of the IBR-000 virus (Cooper strain). The first primer 106.9 (5'-

30 ATGAATTCCCCTGCCGCCGACCGGCACC-3') (SEQ ID NO. 87) sits down on the IBR gpG sequence at amino acid number 1 and introduces an *Eco*RI site at the 5' end of the IBR gpG gene and two additional amino acids between amino acids 1 and 2. The second primer 106.8 (5'-CATGGATCCCGCTCGAGGCGAGCGGGCTCC-

35 3') (SEQ ID NO. 88) sits down on the IBR gpG sequence at

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approximately amino acid 362 on the opposite strand to primer 1 and primes synthesis toward the 5' end of the IBR gpG gene. Fragment 2 was generated by digesting the PCR product with *EcoRI* and *BamHI* to yield a fragment 1085 base pairs in length corresponding to the amino terminal 362 amino acids (approximately 80%) of the IBR gpG gene. Fragment 3 is an approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

HOMOLOGY VECTOR FOR CONSTRUCTING S-SPV-019 (LacZ/IBR gpE HOMOLOGY VECTOR):

This lacZ/IBR gpE homology vector is used to insert foreign DNA into SPV. It incorporates an *E. coli* *B*-galactosidase (*lacZ*) marker gene and the IBR gpE gene flanked by SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the *B*-galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter and the gpE gene is under the control of a synthetic late/early pox promoter. The homology vector may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). The upstream SPV homology is an approximately 1146 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* fragment M (23). The IBR gE gene is an approximately 1888 base pair fragment synthesized by PCR cloning with *EcoRI* and *BamHI* ends. In the PCR cloning procedure, the primers described

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below were used with a template consisting of the IBR-000 VIRUS (Cooper strain). The first primer 4/93.17DR (5'-CTGGTTCGGCCCCAGAATTCTATGGGTCTCGCGGGCTCGTGG-3' (SEQ ID NO. 89) sits down on the IBR gpE gene at amino acid number 1 and  
5 introduces an EcoRI site at the 5' end of the IBR gpE gene and adds two additional amino acids at the amino terminus of the protein. The second primer 4/93.18DR (5' -CTCGCTCGCCCAGGATCCCTAGCGGAGGATGGACTTGAGTCG-3') (SEQ ID NO. 90) sits down on the IBR gpE sequence at approximately amino  
10 acid 648 on the opposite strand to primer 1 and primes synthesis toward the 5' end of the IBR gpE gene. The lacZ promoter and marker gene is identical to the one used in plasmid 520-17.5. The downstream SPV homology is an approximately 2156 base pair AccI to HindIII restriction  
15 sub-fragment of the SPV HindIII restriction fragment M (23). The AccI site in the SPV homology vector is converted to a unique XbaI site.

HOMOLOGY VECTOR FOR CONSTRUCTING S-SPV-018 (LacZ/PRV gpE  
20 HOMOLOGY VECTOR):

This homology vector is constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli B-galactosidase (lacZ) marker gene and the PRV gpE gene  
25 flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR  
30 GENERATING RECOMBINANT SPV, a virus containing the DNA coding for the foreign genes results. Note that the B-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the PRV gpE gene is under the control of a synthetic early/late pox promoter  
35 (EP1LP2). The homology vector is constructed utilizing

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standard recombinant DNA techniques (22,30), by joining restriction fragments from the following sources with synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is the lacZ promoter and marker gene which is identical to the one used in plasmid 520-17.5. Fragment 3 is an approximately 2484 base pair *Dra*I to *Mlu*I sub-fragment of PRV derived from the PRV *Bam*HI #7 DNA fragment. The *Dra*I site is converted to an *Eco*RI site through the use of a synthetic DNA linker. The *Dra*I site sits 45 base pairs upstream of the natural gpE start codon and extends the open reading frame at the amino terminus of the protein for 15 amino acids. The synthetic pox promoter/*Eco*RI DNA linker contributes another 4 amino acids. Therefore, the engineered gpE gene contains 19 additional amino acids fused to the amino terminus of gpE. The nineteen amino acids are Met-Asn-Ser-Gly-Asn-Leu-Gly-Thr-Pro-Ala-Ser-Leu-Ala-His-Thr-Gly-Val-Glu-Thr. Fragment 4 is an approximately 2149 base pair *Acc*I to *Hind*III sub-fragment of the SPV *Hind*III fragment M (23). The *Acc*I sites of fragments 1 and 4 are converted to *Pst*I sites using synthetic DNA linkers.

HOMOLOGY VECTOR 520-90.15. The plasmid 520-90.15 was constructed for the purpose of inserting foreign DNA into SPV. It contains a unique *Nde*I restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the *Nde*I site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign DNA will result. Plasmid 520-90.15 was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining two restriction fragments from the following

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sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second fragment is an approximately 1700 base pair *Hind*III to *Bam*HI restriction subfragment of the  
5 SPV *Hind*III restriction fragment G (23).

HOMOLOGY VECTOR 708-78.9. The plasmid 708-78.9 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*)  
10 marker gene and the infectious bovine rhinotracheitis virus (IBRV) gE gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used  
15 according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the IBRV gE gene is  
20 under the control of a synthetic late/early pox promoter (LP2EP2). It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to  
25 *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl* II to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 475 base pair fragment with *Eco*RI and *Bam*HI restriction sites at the ends. The *Eco*RI and  
30 *Bam*HI sites are synthesized by PCR cloning. The PCR product contains the entire amino acid coding sequence of the IBRV gE gene. In the PCR cloning procedure, the primers described below were used with a template consisting of the IBR-000 virus (Cooper strain) (44). The first primer  
35 2/94.5DR (5'-CTGGTTCGCCCCAGAATTCGATGCAACCCACCGCGCCGCCCCG-3')

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(SEQ ID NO. 116) sits down on the IBR gpE gene at amino acid number 1 and introduces an EcoRI site at the 5' end of the IBRV gE gene and adds two additional amino acids at the amino terminus of the protein. The second primer 4/93.18DR  
5 (5'-CTCGCTCGCCCAGGATCCCTAGCGGAGGATGGACTTGAGTCG-3') (SEQ ID NO. 117) sits down on the IBRV gE sequence (44) at approximately amino acid 648 on the opposite strand to the first primer and primes synthesis toward the 5' end of the IBRV gE gene. The PCR product was digested with EcoRI and  
10 *Bam*HI to yield a fragment approximately 1950 base pairs in length corresponding to the IBRV gE gene. Fragment 3 is an approximately 3002 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *Acc*I to *Hind*III subfragment of  
15 the SPV *Hind*III fragment M. The *Acc*I sites in fragments 1 and 4 were converted to unique *Not*I sites using *Not*I linkers.

HOMOLOGY VECTOR 723-59A9.22. The plasmid 723-59A9.22 was  
20 used to insert foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the equine influenza virus NA PR/56 gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing  
25 DNA coding for the foreign genes results. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1) and the EIV PR/56 NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in  
30 Figures 18A, 18B, 18C and 18D. The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector is derived from an  
35 approximately 2972 base pair *Hind*III to *Bam*HI restriction

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fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl*III to *Acc*I restriction sub-fragment of the SPV *Hind*III fragment M (23). Fragment 2 is the NA gene coding region from the equine Influenza A/Prague/56 (serotype 1 (N7) virus) cloned as an approximately 1450 base pair *Bam*HI fragment utilizing the following primers 5'-  
5 GGGATCCATGAATCCTAATCAAAACTCTTT-3' (SEQ ID NO: 118) for cDNA priming and combined with 5'-  
GGGATCCTTACGAAAAGTATTTAATTTGTGC-3' (SEQ ID NO: 119) for PCR.  
10 (see CLONING OF EQUINE INFLUENZA VIRUS HEMAGGLUTININ AND NEURAMINIDASE GENES). Fragment 3 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *Acc*I to *Hind*III restriction sub-fragment of the SPV *Hind*III  
15 restriction fragment M (23). The *Acc*I site in the SPV homology vector is converted to a unique *Not*I site.

HOMOLOGY VECTOR 727-54.60. The plasmid 727-54.60 was constructed for the purpose of inserting foreign DNA into  
20 SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the pseudorabies virus (PRV) gII (*gpB*) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair  
25 fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late  
30 pox promoter (LP1), and the PRV gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in figures 19A, 19B, 19C, and 19D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction  
35 fragments from the following sources with the synthetic DNA



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sequences indicated in figures 19A to 19D. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 3500 base pair fragment which contains the coding sequence for the PRV gB gene within the *KpnI* C fragment of genomic PRV DNA (21). Fragment 2 contains an approximately 53 base pair synthetic fragment containing the amino terminus of the PRV gB gene, an approximately 78 base pair *SmaI* to *NheI* fragment from the PRV *KpnI* C genomic fragment, and an approximately 3370 base pair *NheI* to *EcoRI* fragment from the PRV *KpnI* C genomic fragment (21). Fragment 3 is an approximately 3010 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

HOMOLOGY VECTOR 727-67.18. The plasmid 727-67.18 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the hepatitis B virus core antigen gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the hepatitis B core antigen gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the plasmid is given in

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figures 20A, 20B, 20C and 20D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figures 20A to 5 20D. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an 10 approximately 3002 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 589 base pair fragment with *BamHI* and *EcoRI* restriction sites at the ends. This fragment contains the hepatitis B core antigen coding sequences (amino acids 25- 15 212) (Ref. 45, 50). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

20 CLONING OF EQUINE INFLUENZA VIRUS HEMAGGLUTININ AND NEURAMINIDASE GENES. The equine influenza virus hemagglutinin (HA) and Neuraminidase (NA) genes may be cloned essentially as described by Katz et al. (42) for the HA gene of human influenza virus. Viral RNA prepared from 25 virus grown in MDBK cells (for Influenza A/equine/Alaska/91 and Influenza A/equine/Miami/63) and MDCK cells (for Influenza A/equine/Prague/56 and Influenza A/equine/Kentucky/81) is first converted to cDNA utilizing an oligo nucleotide primer specific for the target gene. The 30 cDNA is then used as a template for PCR cloning (51) of the targeted gene region. The PCR primers are designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in EHV. One pair of oligo 35 nucleotide primers will be required for each coding region.

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virus AK/91 NA gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes results. Note that the  $\beta$ -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1) and the EIV AK/91 NA gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detail description of the plasmid is given in Figures 21A, 21B, 21C and 21D. The homology vector was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the appropriate synthetic DNA sequences. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* fragment M (23). Fragment 2 is the NA gene coding region from the equine Influenza A/Alaska/91 (serotype 2 (N8) virus) cloned as an approximately 1450 base pair *SalI* fragment utilizing the following primers 5'-GGGTCGACATGAATCCAAATCAAAAGATAA-3' (SEQ ID NO: 124) for cDNA priming and combined with 5'-GGGTCGACTTACATCTTATCGATGTCAAA-3' (SEQ ID NO: 125) for PCR (see CLONING OF EQUINE INFLUENZA VIRUS HEMAGGLUTININ AND NEURAMINIDASE GENES). Fragment 3 is an approximately 3010 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). The *AccI* site in the SPV homology vector is converted to a unique *NotI* site

HOMOLOGY VECTOR 741-80.3 The plasmid 741-80.3 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (lacZ) marker gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV

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DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a human cytomegalovirus immediate early (HCMV IE) promoter. A detailed description of the plasmid is given in figures 22A, 22B and 22C. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figures 22A to 22C. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega).

Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is a 1154 base pair *PstI* to *AvaII* fragment derived from a HCMV 2.1 kb *PstI* fragment containing the HCMV IE promoter (46). Fragment 3 is a 3010 base pair *BamHI* to *PvuII* fragment derived from plasmid pJF751 (49) containing the *E. coli lacZ* gene. Fragment 4 is an approximately 750 base pair *NdeI* to *SalI* fragment derived from PRV *BamHI* #7 which contains the carboxy-terminal 19 amino acids and the polyadenylation signal of the PRV *gX* gene. Fragment 5 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 5 were converted to unique *NotI* sites using *NotI* linkers.

HOMOLOGY VECTOR 741-84.14. The plasmid 741-84.14 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the human interleukin-2 (IL-2) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream

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of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the human IL-2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). The coding sequence for the human IL-2 protein is fused at the amino terminus to the PRV gX signal sequence for membrane transport. A detailed description of the plasmid is given in figures 23A, 23B, 23C, and 23D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figures 23A to 23D. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglIII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 475 base pair fragment with *EcoRI* and *BglIII* restriction sites at the ends. The *EcoRI* site is synthesized by PCR cloning and the *BglIII* site is at the 3' end of the human IL-2 cDNA (43, 47). The PCR product contains the entire amino acid coding sequence of the PRV gX signal sequence-human IL-2 gene. In this procedure, the primers described below were used with a template consisting of the cDNA for PRV gX signal sequence-human IL-2 (43). The first primer 5/94.23 (5'-CTCGAATTCGAAGTGGGCAACGTGGATCCTCGC-3') (SEQ ID NO 126) sits down on the PRV gX signal sequence at amino acid number 1 and introduces an *EcoRI* site at the 5' end of the gene. The second primer 5/94.24 (5'-CAGTTAGCCTCCCCCATCTCCCCA-3') (SEQ ID NO. 127) sits down on the human IL-2 gene sequence within the 3' untranslated region on the opposite strand to primer 5/94.23 and primes

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toward the 5' end of the gene. The PCR product was digested with *EcoRI* and *BglII* (*BglII* is located approximately 3 nucleotides beyond the stop codon for the human IL-2 gene) to yield a fragment 475 base pairs in length corresponding to the PRV gX signal sequence-human IL-2 gene. Fragment 3 is an approximately 3010 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

HOMOLOGY VECTOR 744-34. The plasmid 744-34 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the equine herpesvirus type 1 gB gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the EHV-1 gB gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in figures 24A, 24B, 24C and 24D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figures 24A to 24D. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *BglII* to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23).

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Fragment 2 is an approximately 2941 base pair fragment with EcoRI and PmeI restriction sites at the ends. Fragment 2 is an approximately 2941 base pair EcoRI to PmeI fragment. Fragment 2 was synthesized as an approximately 429 base pair PCR fragment at the 5' end of the gene having a synthetic EcoRI site and a natural BamHI site within the BamHI "a" fragment of EHV-1 genomic DNA and an approximately 2512 base pair restriction fragment at the 3' end of the gene from BamHI to PmeI within the BamHI "i" fragment of EHV-1 genomic DNA (48). In the procedure to produce the 5' end PCR fragment, the primers described below were used with a template consisting of the EHV-1 BamHI "a" and "i" fragments. The first primer 5/94.3 (5'-CGGAATTCCTCTGGTTGCCGT-3') (SEQ ID NO 128) sits down on the EHV-1 gB sequence at amino acid number 2 and introduces an EcoRI site at the 5' end of the EHV-1 gB gene and an ATG start codon. The second primer 5/94.4 (5'-GACGGTGGATCCGGTAGGCGGT-3') (SEQ ID NO. 129) sits down on the EHV-1 gB sequence at approximately amino acid 144 on the opposite strand to primer 5/94.3 and primes toward the 5' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment 429 base pairs in length corresponding to the 5' end of the EHV-1 gB gene. Fragment 2 consists of the products of the PCR reaction (EcoRI to BamHI) and the restriction fragment (BamHI to PmeI) ligated together to yield an EHV-1 gB gene which is an EcoRI to PmeI fragment approximately 2941 base pairs (979 amino acids) in length. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

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HOMOLOGY VECTOR 744-38. The plasmid 744-38 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli*  $\beta$ -galactosidase (*lacZ*) marker gene and the equine herpesvirus type 1 gD gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter (LP1), and the EHV-1 gD gene is under the control of a synthetic late/early pox promoter (LP2EP2). A detailed description of the plasmid is given in figures 25A, 25B, 25C and 25D. It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figures 25A to 25D. The plasmid vector is derived from an approximately 2972 base pair *HindIII* to *BamHI* restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair *Bgl* II to *AccI* restriction sub-fragment of the SPV *HindIII* restriction fragment M (23). Fragment 2 is an approximately 1240 base pair *HindIII* fragment within the *BamHI* "d" fragment of EHV-1 (48). Fragment 3 is an approximately 3010 base pair *BamHI* to *PvuII* restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair *AccI* to *HindIII* subfragment of the SPV *HindIII* fragment M. The *AccI* sites in fragments 1 and 4 were converted to unique *NotI* sites using *NotI* linkers.

CLONING OF PARAINFLUENZA-3 VIRUS FUSION AND HEMAGGLUTININ GENES. The parainfluenza-3 virus fusion (F) and hemagglutinin (HN) genes were cloned by a PCR CLONING



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procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA prepared from bovine PI-3 virus grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The F gene coding region from the PI-3 strain SF-4 (VR-281) was cloned using the following primers: 5'-TTATGGATCCTGCTGCTGTGTTGAACAACCTTTGT-3' (SEQ ID NO: 130) for cDNA priming and combined with 5'-CCGCGGATCCCATGACCATCACAAACCATAATCATAGCC-3' (SEQ ID NO: 131) for PCR. The HN gene coding region from PI-3 strain SF-4 (VR-281) was cloned utilizing the following primers: 5'-CGTCGGATCCCTTAGCTGCAGTTTTTTGGAACTTCTGTTTTGA-3' (SEQ ID NO: 132) for cDNA priming and combined with 5'-CATAGGATCCCATGGAATATTGGAACACACAAACAGCAC-3' (SEQ ID NO: 133) for PCR. Note that this general strategy is used to clone the coding region of F and HN genes from other strains of PI-3. The DNA fragment for PI-3 HN or F was digested with *Bam*HI to yield an 1730 bp or 1620 bp fragment, respectively. The PI-3 HN fragment is cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector. The PI-3 F fragment is cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vector 713-55.10.

CLONING OF BOVINE VIRAL DIARRHEA VIRUS gp48 and gp53 GENES. The bovine viral diarrhea gp48 and gp53 genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human influenza. Viral RNA

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prepared from BVD virus Singer strain grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in SPV. One pair of oligonucleotides were required for each coding region. The gp48 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5' - ACGTCGGATCCCTTACCAAACCACGTCTTACTCTTGTTC-3' (SEQ ID NO: 134) for cDNA priming and combined with 5' - ACATAGGATCCCATGGGAGAAAACATAACACAGTGAACC-3' (SEQ ID NO: 135) for PCR. The gp53 gene coding region from the BVDV Singer strain (49) was cloned using the following primers: 5' - CGTGGATCCTCAATTACAAGAGGTATCGTCTAC-3' (SEQ ID NO: 136) for cDNA priming and combined with 5' - CATAGATCTTGTGGTGCTGTCCGACTTCGCA-3' (SEQ ID NO: 137) for PCR. Note that this general strategy is used to clone the coding region of gp48 and gp53 genes from other strains of BVDV. The DNA fragment for BVDV gp 48 was digested with *Bam*HI to yield an 678 bp fragment. The DNA fragment for BVDV gp 53 was digested with *Bgl*III and *Bam*HI to yield an 1187 bp fragment. The BVDV gp48 or gp53 DNA fragments were cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-78.1 and 738-96, respectively.

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CLONING OF BOVINE RESPIRATORY SYNCYTIAL VIRUS FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. The bovine respiratory syncytial virus fusion (F), nucleocapsid (N), and glycoprotein (G) genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for

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the HA gene of human influenza. Viral RNA prepared from BRSV virus grown in bovine nasal turbinate (BT) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target gene. The cDNA was then used as a

5 template for polymerase chain reaction (PCR) cloning (15) of the targeted region. The PCR primers were designed to incorporate restriction sites which permit the cloning of the amplified coding regions into vectors containing the appropriate signals for expression in SPV. One pair of

10 oligonucleotides were required for each coding region. The F gene coding region from the BRSV strain 375 (VR-1339) was cloned using the following primers: 5'-TGCAGGATCCTCATTACTAAAGGAAAGATTGTTGAT-3' (SEQ ID NO: 138) for cDNA priming and combined with 5'-

15 CTCTGGATCCTACAGCCATGAGGATGATCATCAGC-3' (SEQ ID NO: 139) for PCR. The N gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5'-CGTCGGATCCCTCACAGTTCACATCATTGTCTTTGGGAT-3' (SEQ ID NO: 140) for cDNA priming and combined with 5'-

20 CTTAGGATCCCATGGCTCTTAGCAAGGTCAAACATAATGAC-3' (SEQ ID NO: 141) for PCR. The G gene coding region from BRSV strain 375 (VR-1339) was cloned utilizing the following primers: 5'-CGTTGGATCCCTAGATCTGTGTAGTTGATTGATTTGTGTGA-3' (SEQ ID NO: 142) for cDNA priming and combined with 5'-

25 CTCTGGATCCTCATACCCATCATCTTAAATTCAAGACATTA-3' (SEQ ID NO: 143) for PCR. Note that this general strategy is used to clone the coding region of F, N and G genes from other strains of BRSV. The DNA fragments for BRSV F, N, or G were digested with *Bam*HI to yield 1722 bp, 1173 bp, or 771 bp

30 fragments, respectively. The BRSV F, N, and G DNA fragments were cloned into the *Bam*HI site next to the LP2EP2 promoter of the SPV homology vector to yield homology vectors, 727-20.10, 713-55.37 and 727-20.5, respectively.

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HOMOLOGY VECTOR 689-50.4 The plasmid 689-50.4 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli  $\beta$ -galactosidase (lacZ) marker gene and the infectious bursal disease virus (IBDV) polyprotein gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the IBDV polyprotein gene is under the control of a synthetic late/early pox promoter (LP2EP2). It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector is derived from an approximately 2972 base pair Hind III to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglIII to AccI restriction subfragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 3400 base pair fragment with SmaI and HpaI restriction sites at the ends from plasmid 2-84/2-40 (51). This fragment contains the IBDV polyprotein coding sequences. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149 base pair AccI to HindIII subfragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

HOMOLOGY VECTOR 689-50.7. The plasmid 689-50.7 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli  $\beta$ -galactosidase (lacZ)

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marker gene and the infectious bursal disease virus (IBDV) VP2 gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 2149  
5 base pair fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a virus containing DNA coding for the foreign genes will result. Note that the  $\beta$ -galactosidase (lacZ) marker gene is under the control of a  
10 synthetic late pox promoter (LP1), and the IBDV VP2 gene is under the control of a synthetic late/early pox promoter (LP2EP2). It may be constructed utilizing standard recombinant DNA techniques (22, 30), by joining restriction fragments from the following sources. The plasmid vector is  
15 derived from an approximately 2972 base pair HindIII to BamHI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 1484 base pair BglIII to AccI restriction sub-fragment of the SPV HindIII restriction fragment M (23). Fragment 2 is an approximately 1081 base pair fragment with  
20 BclI and BamHI restriction sites at the ends. This fragment codes for the IBDV VP2 protein and is derived from a full length IBDV cDNA clone (51). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 2149  
25 base pair AccI to HindIII sub-fragment of the SPV HindIII fragment M. The AccI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

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EXAMPLESExample 1

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Homology Vector 515-85.1. The homology vector 515-85.1 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 515-85.1 contains a unique *AccI* restriction site into which foreign DNA may be cloned. A plasmid containing  
10 such a foreign DNA insert may be used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful it is important that the insertion site (*AccI*) be in a region non-essential  
15 to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. We have demonstrated that the *AccI* site in homology vector 515-85.1 may be used to insert foreign DNA into at least three  
20 recombinant SPV (see examples 2-4).

In order to define an appropriate insertion site, a library of SPV *HindIII* restriction fragments was generated. Several of these restriction fragments (*HindIII* fragments G, J, and  
25 M see figures 1A and 1B) were subjected to restriction mapping analysis. Two restriction sites were identified in each fragment as potential insertion sites. These sites included *HpaI* and *NruI* in fragment G, *BalI* and *XbaI* in fragment J, and *AccI* and *PstI* in fragment M. A  $\beta$ -galactosidase (*lacZ*) marker gene was inserted in each of the  
30 potential sites. The resulting plasmids were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The generation of recombinant virus was determined by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -  
35 GALACTOSIDASE ASSAYS. Four of the six sites were found to

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generate recombinant virus, however the ability of each of these viruses to be purified away from the parental SPV varied greatly. In one case virus could not be purified above the level of 1%, in another case virus could not be purified above the level of 50%, and in a third case virus could not be purified above the level of 90%. The inability to purify these viruses indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the AccI site of Homology vector 515-85.1, resulted in a virus which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

The homology vector 515-85.1 was further characterized by DNA sequence analysis. Two regions of the homology vector were sequenced. The first region covers a 599 base pair sequence which flanks the unique AccI site (see figures 2A and 2B). The second region covers the 899 base pairs upstream of the unique HindIII site (see figures 2A and 2B). The sequence of the first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the vaccinia virus (VV) O1L open reading frame identified by Goebel *et al.*, 1990 (see figures 3A, 3B and 3C). The sequence of the second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same vaccinia virus O1L open reading frame (see figures 3A, 3B and 3C). These data suggest that the AccI site interrupts the presumptive VV O1L-like ORF at approximately amino acid 41, suggesting that this ORF codes for a gene non-essential for SPV replication. Goebel *et al.* suggest that the VV O1L ORF contains a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for virus replication.

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The DNA sequence located upstream of the VV 01L-like ORF (see Figure 2A) would be expected to contain a swinepox viral promoter. This swinepox viral promoter will be useful as the control element of foreign DNA introduced into the swinepox genome.

### Example 2

#### S-SPV-003

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S-SPV-003 is a swinepox virus that expresses a foreign gene.

The gene for *E.coli*  $\beta$ -galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The foreign gene (lacZ) is under the control of a synthetic early/late promoter (EP1LP2).

15

S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-003. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the foreign gene. The assays described here were carried out in VERO cells as well as EMSK cells, indicating that VERO cells would be a suitable substrate for the production of SPV recombinant vaccines. S-SPV-003 has been deposited with the ATCC under Accession No. VR 2335.

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Example 3S-SPV-008

5 S-SPV-008 is a swinepox virus that expresses at least two foreign genes. The gene for E. coli  $\beta$ -galactosidase (lacZ gene) and the gene for pseudorabies virus (PRV) g50 (gpD) (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late promoter (LP1) and  
10 the g50 (gp)D gene is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16  
15 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque  
20 purification was the recombinant virus designated S-SPV-008. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification,  
25 all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

S-SPV-008 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE  
30 EXPRESSION IN RECOMBINANT SPV. Swine anti-PRV serum was shown to react specifically with S-SPV-008 plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum indicating that the virus was stably expressing the PRV foreign gene.  
35 The black plaque assay was also performed on unfixed

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monolayers. The SPV plaques on the unfixed monolayers also exhibited specific reactivity with swine anti-PRV serum indicating that the PRV antigen is expressed on the infected cell surface.

5

To confirm the expression of the PRV g50 (gpD) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the  
10 WESTERN BLOTTING PROCEDURE. The swine anti-PRV serum was used to detect expression of PRV specific proteins. As shown in figure 6, the lysate from S-SPV-008 infected cells exhibits a specific band of approximately 48 kd, the reported size of PRV g50 (gpD) (35).

15

PRV g50 (gpD) is the g50 (gpD) homologue of HSV-1 (26). Several investigators have shown that VV expressing HSV-1 g50 (gpD) will protect mice against challenge with HSV-1 (6 and 34). Therefore the S-SPV-008 should be valuable as a  
20 vaccine to protect swine against PRV disease.

It is anticipated that several other PRV glycoproteins will be useful in the creation of recombinant swinepox vaccines to protect against PRV disease. These PRV glycoproteins  
25 include gpII (28), gpIII (27), and gpH (19). The PRV gpIII coding region has been engineered behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox viruses expressing all four of these PRV glycoprotein genes.  
30 Such recombinant swinepox viruses will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated

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011 plaques and not with S-SPV-001 negative control plaques. All S-SPV-011 observed plaques reacted with the swine anti-PRV serum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in EMSK cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the PRV gIII (gpC) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gpC) antibody was used to detect expression of PRV specific proteins. As shown in figure 16, the lysate from S-SPV-011 infected cells exhibits a specific band of approximately 92 kd, the reported size of PRV gIII (gpC) (37).

Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection from challenge with virulent PRV is obtained (39). Therefore S-SPV-011 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

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Example 5S-SPV-012

5 S-SPV-012 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* B-galactosidase (*lacZ*) and the gene for pseudorabies virus gIII (*gpC*) were inserted into the unique *Pst*I restriction site (*Pst*I linkers inserted into a unique *Acc*I site) of the homology vector 570-33.32.  
10 The *lacZ* gene is under the control of the synthetic late promoter (LP1) and the PRV gIII (*gpC*) gene is under the control of the synthetic early late promoter (EP1LP2).

S-SPV-012 was derived from S-SPV-001 (Kasza Strain). This  
15 was accomplished utilizing the homology vector 570-91.41 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE  
20 (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-012. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and  
25 Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-012 was assayed for expression of PRV specific  
30 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-PRV gIII (*gpC*) antibody was shown to react specifically with S-SPV-012 plaques and not with S-SPV-001 negative control plaques. All S-SPV-012 observed plaques reacted with the swine anti-  
35 PRV serum, indicating that the virus was stably expressing

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the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

5

To confirm the expression of the PRV gIII (gpC) gene product, cells were infected with S-SPV-012 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gpC) antibody was used to detect expression of PRV specific proteins. As shown in figure 16, the lysate from S-SPV-012 infected cells exhibits two specific bands which are the reported size of PRV gIII (gpC) (37) - a 92 kd mature form and a 74 kd pre-golgi form.

10  
15

Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection from challenge with virulent PRV is obtained (39). Therefore S-SPV-012 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals.

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Example 6S-SPV-013

- 5 S-SPV-013 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* B-galactosidase (*lacZ*) and the gene for pseudorabies virus *gIII* (*gpC*) were inserted into the unique *Pst*I restriction site (*Pst*I linkers inserted into a unique *Acc*I site) of the homology vector 570-33.32.
- 10 The *lacZ* gene is under the control of the synthetic late promoter (LP1) and the PRV *gIII* (*gpC*) gene is under the control of the synthetic late early promoter (LP2EP2).

- S-SPV-013 was derived from S-SPV-001 (Kasza Strain). This
- 15 was accomplished utilizing the homology vector 570-91.64 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE
- 20 (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-013. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and
- 25 Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

- S-SPV-013 was assayed for expression of PRV specific
- 30 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal goat anti-PRV *gIII* (*gpC*) antibody was shown to react specifically with S-SPV-013 plaques and not with S-SPV-001 negative control plaques. All S-SPV-013 observed plaques reacted with the swine anti-
- 35 PRV serum indicating that the virus was stably expressing

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the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating that EMSK cells would be a suitable substrate for the production of SPV recombinant vaccines.

5

To confirm the expression of the PRV gIII (gpC) gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal goat anti-PRV gIII (gpC) antibody was used to detect expression of PRV specific proteins. As shown in figure 16, the lysate from S-SPV-013 infected cells exhibits two specific bands which are the reported size of PRV gIII (gpC) (37)-a 92 kd mature form and a 74 kd pre-Golgi form.

15

Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant immune response in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection from challenge with virulent PRV is obtained. (39) Therefore S-SPV-013 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-013 has been deposited with the ATCC under Accession No. \_\_\_\_\_.

25

Protection against Aujeszky's disease using recombinant swinepox virus vaccines S-SPV-008 and S-SPV-013.

30

A vaccine containing S-SPV-008 and S-SPV-013 ( $1 \times 10^6$  PFU/ml) (2ml of a 1:1 mixture of the two viruses) was given to two groups of pigs (5 pigs per group) by intradermal inoculation

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or by oral/pharyngeal spray. A control group of 5 pigs received S-SPV-001 by both intradermal and oral/pharyngeal inoculation. Pigs were challenged three weeks post-vaccination with virulent PRV, strain 4892, by intranasal inoculation. The table presents a summary of clinical responses. The data support an increase in protection against Aujeszky's disease in the S-SPV-008/S-SPV-013 vaccinates compared to the S-SPV-001 vaccinate controls.

10	Vaccine	Route of inoculation	Post-challenge Respiratory Signs: (# with signs/ total number)	Post-challenge CNS signs: (# with signs/ total number)	Post-challenge Group average: (Days of clinical signs)
	S-SPV-008 + S-SPV-013	Intradermal	3/5	0/5	2.6
	S-SPV-008 + S-SPV-013	Oral/ pharyngeal	3/5	0/5	2.2
15	S-SPV-001 (Control)	Intradermal + Oral/ Pharyngeal	5/5	2/5	7.8

#### Example 7

20

#### S-SPV-015

S-SPV-015 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for pseudorabies virus (PRV) gII (gpB) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restrict

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ion site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRV gB gene is under the control of the synthetic late/early promoter (LP2EP2).

5 S-SPV-015 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-54.60 (see Materials and Methods) and virus S-SPV-001 in the  
10 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-015. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored  
15 by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 S-SPV-015 was assayed for expression of PRV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal swine anti-PRV serum was shown to react specifically with S-SPV-015 plaques and not with S-SPV-001 negative control plaques. All S-SPV-  
25 015 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

30

To confirm the expression of the PRV gII gene product, cells were infected with SPV-015 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the  
35 WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-PRV serum

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was used to detect expression of PRV specific proteins. The lysate from S-SPV-015 infected cells exhibited bands corresponding to 120 kd, 67 kd and 58 kd, which are the expected size of the PRV gII glycoprotein.

5

S-SPV-015 is useful as a vaccine in swine against pseudorabies virus. A superior vaccine is formulated by combining S-SPV-008 (PRV g50), S-SPV-013 (PRV gIII), and S-SPV-015 for protection against pseudorabies in swine.

10

Therefore S-SPV-015 should be valuable as a vaccine to protect swine against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-015 has been deposited with the ATCC under Accession No.

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#### 20 Example 8

Recombinant swinepox virus expressing more than one pseudorabies virus (PRV) glycoproteins, which can elicit production of neutralizing antibodies against pseudorabies virus, is constructed in order to obtain a recombinant swinepox virus with enhanced ability to protect against PRV infection than that which can be obtained by using a recombinant swinepox virus expressing only one of those PRV glycoproteins.

25  
30

There are several examples of such recombinant swinepox virus expressing more than one PRV glycoproteins: a recombinant swinepox virus expressing PRV g50 (gpD) and gIII (gpC), a recombinant swinepox virus expressing PRV g50 (gpD) and gII (gpB); a recombinant swinepox virus expressing PRV

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gII (gpB) and gIII (gpC); and a recombinant swinepox virus expressing PRV g50 (gpD), gIII (gpC) and gII (gpB). Each of the viruses cited above is also engineered to contain and express *E. coli* B-galactosidase (lac Z) gene, which will facilitate the cloning of the recombinant swinepox virus.

Listed below are three examples of a recombinant swinepox virus expressing PRV g50 (gpD), PRV gIII (gpC), PRV gII (gpB) and *E. coli* B-galactosidase (lacZ):

10

a) Recombinant swinepox virus containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacZ gene. All four genes are inserted into the unique *AccI* restriction endonuclease site within the *HindIII* M fragment of the swinepox virus genome. PRV g50 (gpD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is under the control of a synthetic early promoter (EP2), PRV gII (gpB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

20

b) Recombinant swinepox virus containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacZ gene. All four genes are inserted into the unique *AccI* restriction endonuclease site within the *HindIII* M fragment of the swinepox virus genome. PRV g50 (gpD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gII (gpB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

30

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c) Recombinant swinepox virus containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacZ gene. All four genes are inserted into the unique AccI restriction endonuclease site within the *HindIII* M fragment of the swinepox virus genome. PRV g50 (gpD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is under the control of a synthetic late/early promoter (LP2EP2), PRV gII (gpB) gene is under the control of a synthetic late/early promoter (LP2EP2) and lacZ gene is under the control of a synthetic late promoter (LP1).

#### Example 9

##### S-SPV-009

S-SPV-009 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ gene) and the gene for Newcastle's Disease virus hemagglutinin (HN) gene were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late promoter (LP1) and the HN gene is under the control of an synthetic early/late promoter (EP1LP2).

S-SPV-009 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.26 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-009. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored

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by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

5

S-SPV-009 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rabbit anti-NDV HN serum was shown to react specifically with S-SPV-009 plaques and not with S-SPV-008 negative control plaques. All S-SPV-009 observed plaques reacted with the swine antiserum indicating that the virus was stably expressing the NDV foreign gene. S-SPV-009 has been deposited with the ATCC under Accession No. VR 2344).

15

To confirm the expression of the NDV HN gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The rabbit anti-NDV HN serum was used to detect expression of the HN protein. The lysate from S-SPV-009 infected cells exhibited a specific band of approximately 74 kd, the reported size of NDV HN (29).

25 Example 10S-SPV-014

S-SPV-014 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* B-galactosidase (lacZ) and the gene for infectious laryngotracheitis virus glycoprotein G (ILT gpG) were inserted into the SPV 570-33.32 ORF (a unique PstI site has replaced the unique AccI site). The lacZ gene is under the control of the synthetic

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late promoter (LP1), and the ILT gpG gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-014 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 599-65.25 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-014. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the ILT gpG gene product, cells were infected with SPV-014 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Peptide antisera to ILT gpG was used to detect expression of ILT specific proteins. The lysate from S-SPV-014 infected cells exhibited a band at 43 kd which is the expected size of the ILT gpG protein and additional bands of higher molecular weight which represent glycosylated forms of the protein which are absent in deletion mutants for ILT gpG.

This virus is used as an expression vector for expressing ILT glycoprotein G (gpG). Such ILT gpG is used as an

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antigen to identify antibodies directed against the wild-type  
ILT virus as opposed to antibodies directed against gpG  
deleted ILT viruses. This virus is also used as an antigen  
for the production of ILT gpG specific monoclonal  
5 antibodies. Such antibodies are useful in the development  
of diagnostic tests specific for the ILT gpG protein.  
Monoclonal antibodies are generated in mice utilizing this  
virus according to the PROCEDURE FOR PURIFICATION OF VIRAL  
GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

10

Example 11

## S-SPV-016

15 S-SPV-016 is a swinepox virus that expresses at least two  
foreign genes. The gene for *E. coli* B-galactosidase (lacZ)  
and the gene for infectious laryngotracheitis virus  
glycoprotein I (ILT gpI) were inserted into the SPV 617-48.1  
ORF (a unique NotI restriction site has replaced a unique  
20 AccI restriction site). The lacZ gene is under the control  
of the synthetic late promoter (LP1), and the ILT gpI gene  
is under the control of the synthetic late/early promoter  
(LP2EP2).

25 S-SPV-016 was derived from S-SPV-001 (Kasza Strain). This  
was accomplished utilizing the homology vector 624-20.1C  
(see Materials and Methods) and virus S-SPV-001 in the  
HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING  
RECOMBINANT SPV. The transfection stock was screened by the  
30 SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE  
(BLUOGAL AND CPRG ASSAYS). The final result of red plaque  
purification was the recombinant virus designated S-SPV-016.  
This virus was assayed for B-galactosidase expression,  
purity, and insert stability by multiple passages monitored  
35 by the blue plaque assay as described in Materials and

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Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-016 was assayed for expression of ILT gpI- and B-galactosidase-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Polyclonal chicken anti-ILT antibody was shown to react specifically with S-SPV-016 plaques and not with S-SPV-017  
10 negative control plaques. All S-SPV-016 observed plaques reacted with the chicken antiserum indicating that the virus was stably expressing the ILT foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the  
15 production of SPV recombinant vaccines.

To confirm the expression of the ILT gpI gene product, cells were infected with SPV-016 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel  
20 electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal chicken anti-ILT antibody was used to detect expression of ILT specific proteins. The lysate from S-SPV-016 infected cells exhibits a range of bands reactive to the anti-ILT antibody from 40  
25 to 200 kd indicating that the ILT gpI may be heavily modified.

This virus is used as an expression vector for expressing ILT glycoprotein I (gpI). Such ILT gpI is used as an  
30 antigen to identify antibodies directed against the wild-type ILT virus as opposed to antibodies directed against gpI deleted ILT viruses. This virus is also used as an antigen for the production of ILT gpI specific monoclonal antibodies. Such antibodies are useful in the development  
35 of diagnostic tests specific for the ILT gpI protein.



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Monoclonal antibodies are generated in mice utilizing this virus according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

5     Example 12

S-SPV-017

10     S-SPV-017 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* B-galactosidase (lacZ) and the gene for infectious bovine rhinotracheitis virus glycoprotein G (IBR gpG) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control  
15     of the synthetic late promoter (LP1), and the IBR gpG gene is under the control of the synthetic late/early promoter (LP2EP2).

20     S-SPV-017 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 614-83.18 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE  
25     (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-017. This virus was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and  
30     Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

35     S-SPV-017 was assayed for expression of IBR-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE

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EXPRESSION IN RECOMBINANT SPV. Monoclonal antibodies and peptide antisera to IBR gpG were shown to react specifically with S-SPV-017 plaques and not with S-SPV-016 negative control plaques. All S-SPV-017 observed plaques reacted with the antiserum indicating that the virus was stably expressing the IBR foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

10

To confirm the expression of the IBR gpG gene product, cells were infected with SPV-017 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Antisera to IBR gpG was used to detect expression of IBR specific proteins. The lysate from S-SPV-017 infected cells exhibited a band at 43 kd which is the expected size of the IBR gpG protein and additional bands of higher molecular weight which represent glycosylated forms of the protein which are absent in deletion mutants for IBR gpG.

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This virus is used as an expression vector for expressing IBR glycoprotein G (gpG). Such IBR gpG is used as an antigen to identify antibodies directed against the wild-type IBR virus as opposed to antibodies directed against gpG deleted IBR viruses. This virus is also used as an antigen for the production of IBR gpG specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the IBR gpG protein. Monoclonal antibodies are generated in mice utilizing this virus according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

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Example 13S-SPV-019

5 S-SPV-019 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for infectious bovine rhinotracheitis virus (IBRV) gE were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the IBRV gE gene is under the control of the synthetic late/early promoter (LP2EP2).

15 S-SPV-019 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 708-78.9 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-019. This virus was assayed for  $\beta$ -galactosidase expression, purity and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

30 This virus is used as an expression vector for expressing IBR glycoprotein E (gpE). Such IBR gpE is used as an antigen to identify antibodies directed against the wild-type IBR virus as opposed to antibodies directed against gpE deleted IBR viruses. This virus is also used as an antigen for the production of IBR gpE specific monoclonal antibodies. Such antibodies are useful in the development

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of diagnostic tests specific for the IBR gpE protein. Monoclonal antibodies are generated in mice utilizing this virus according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

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Example 14S-SPV-018

10 S-SPV-018 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for pseudorabies virus glycoprotein E (PRV gpE) are inserted into the SPV 570-33.32 ORF (a unique PstI site has replaced the unique AccI site). The lacZ gene is under  
15 the control of the synthetic late promoter (LP1), and the PRV gpE gene is under the control of the synthetic early/late promoter (EP1LP2).

S-SPV-018 is derived from the S-SPV-001 (Kasza Strain).  
20 This is accomplished utilizing the final homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). Red plaque  
25 purification of the recombinant virus is designated S-SPV-018. This virus is assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay described in Materials and Methods. After the initial three rounds of purification, all plaques  
30 observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

This virus is used as an expression vector for expressing PRV glycoprotein E (gpE). Such PRV gpE is used as an  
35 antigen to identify antibodies directed against the wild-

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type PRV virus as opposed to antibodies directed against gpE deleted PRV viruses. This virus is also used as an antigen for the production of PRV gpE specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the PRV gpE protein. Monoclonal antibodies are generated in mice utilizing this virus according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

10 Example 15

Homology Vector 520-90.15

The homology vector 520-90.15 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 520-90.15 contains a unique NdeI restriction site into which foreign DNA may be cloned. A plasmid containing such a foreign DNA insert has been used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful, it is important that the insertion site be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The unique NdeI restriction site in plasmid 520-90.15 is located within the coding region of the SPV thymidine kinase gene (32). Therefore, we have shown that the thymidine kinase gene of swinepox virus is non-essential for DNA replication and is an appropriate insertion site.

30 Example 16

S-PRV-010

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S-SPV-010 is a swinepox virus that expresses a foreign gene. The *E. coli* *B*-galactosidase (*lacZ*) gene is inserted into a unique *Nde*I restriction site within the thymidine kinase gene. The foreign gene (*lacZ*) is under the control of the  
5 synthetic late promoter, LP1. We have shown that the swinepox virus thymidine kinase gene is non-essential for replication of the virus and is an appropriate insertion site.

10 A 1739 base pair *Hind*III-BamHI fragment subcloned from the *Hind*III G fragment contains the swinepox virus thymidine kinase gene and is designated homology vector 520-90.15. The homology vector 520-90.15 was digested with *Nde* I, and *Asc*I linkers were inserted at this unique site within the  
15 thymidine kinase gene. The LP1 promoter-lac Z cassette with *Asc*I linkers was ligated into the *Asc* I site within the thymidine kinase gene. The recombinant homology vector 561-36.26 was cotransfected with virus S-SPV-001 by the  
20 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV and virus plaques expressing *B*-galactosidase were selected by SCREEN FOR RECOMBINANT SPV EXPRESSING *B*-GALACTOSIDASE (BLUOGAL AND CPRG ASSAY). The final result of blue and red plaque purification was the recombinant virus designated S-SPV-010. This virus was assayed for *B*-  
25 galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing  
30 the foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

35 Example 17

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The development of vaccines utilizing the swinepox virus to express antigens from various disease causing microorganisms can be engineered.

#### 5 TRANSMISSIBLE GASTROENTERITIS VIRUS

The major neutralizing antigen of the transmissible gastroenteritis virus (TGE), glycoprotein 195, for use in the swinepox virus vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S. Serial No. 10 078,519, filed July 27, 1987. It is contemplated that the procedures that have been used to express PRV g50 (gpD) in SPV and are disclosed herein are applicable to TGE.

#### PORCINE PARVOVIRUS

15 We have cloned the major capsid protein of the porcine (swine) parvovirus (PPV) for use in the swinepox virus vector. The clone of the capsid protein is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to 20 express PRV g50 (gpD) in SPV and are disclosed herein are applicable to PPV.

#### SWINE ROTAVIRUS

We have cloned the major neutralizing antigen of the swine 25 rotavirus, glycoprotein 38, for use in the swinepox virus vector. The clone of glycoprotein 38 is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV g50 (gpD) in SPV and are disclosed herein are 30 applicable to SRV.

#### HOG CHOLERA VIRUS

The major neutralizing antigen of the bovine viral diarrhea (BVD) virus was cloned as disclosed in U.S. Serial No. 35 225,032, filed July 27, 1988. Since the BVD and hog cholera

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viruses are cross protective (31), the BVD virus antigen has been targeted for use in the swinepox virus vector. It is contemplated that the procedures that have been used to express PRV g50 (gpD) in SPV and are disclosed herein are applicable to BVD virus.

#### *SERPULINA HYODYSENTERIAE*

A protective antigen of *Serpulina hyodysenteriae* (3), for use in the swinepox virus vector has been cloned. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are also applicable to *Serpulina hyodysenteriae*.

Antigens from the following microorganisms may also be utilized to develop animal vaccines: swine influenza virus, foot and mouth disease virus, African swine fever virus, hog cholera virus, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome/swine infertility and respiratory syndrome (PRRS/SIRS).

Antigens from the following microorganisms may also be utilized to develop animal vaccines: feline leukemia virus, feline immunodeficiency virus, feline herpesvirus, feline infectious peritonitis virus, canine herpesvirus, canine coronavirus, canine parvovirus, parasitic diseases in animals (including *Dirofilaria immitis* in dogs and cats), equine infectious anemia, *Streptococcus equi*, coccidia, emeria, chicken anemia virus, *Borrelia bergdorferi*, bovine coronavirus, pasteurella, haemolytica

#### Example 18

Recombinant swinepox viruses express equine influenza virus type A/Alaska 91, equine influenza virus type A/Prague 56, equine herpesvirus type 1 gB, or equine herpesvirus type 1



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gD genes. S-SPV-033 and S-SPV-034 are useful as vaccines against equine influenza infection, and S-SPV-038 and S-SPV-039 are useful as a vaccine against equine herpesvirus infection which causes equine rhinotracheitis and equine abortion. These equine influenza and equine herpesvirus antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. The swinepox virus is useful for cloning other subtypes of equine influenza virus (including EIVA/Miami/63 and EIVA/Kentucky/81) to protect against rapidly evolving variants in this disease. S-SPV-033, S-SPV-034, S-SPV-038, and S-SPV-039 are also useful as an expression vector for expressing equine influenza or equine herpesvirus antigens. Such equine influenza or equine herpesvirus antigens are useful to identify antibodies directed against the wild-type equine influenza virus or equine herpesvirus. The viruses are also useful to in producing antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

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Example 18AS-SPV-033:

30 S-SPV-033 is a recombinant swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for equine influenza virus type A/Alaska 91 neuraminidase were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a  
35 unique AccI restriction site). The lacZ gene is under the

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control of the synthetic late promoter (LP1), and the EIV AK/91 NA gene is under the control of the synthetic late/early promoter (LP2EP2).

5 S-SPV-033 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 732-18.4 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR  
10 RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-033. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque  
15 assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 Example 18B

S-SPV-034:

S-SPV-034 is a swinepox virus that expresses at least two  
25 foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for equine influenza virus type A/Prague 56 neuraminidase were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the  
30 synthetic late promoter (LP1), and the EIV PR/56 NA gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-034 was derived from S-SPV-001 (Kasza Strain). This  
35 was accomplished utilizing the homology vector 723-59A9.22

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(see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase  
5 (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-034. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and  
10 Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-034 was assayed for expression of EIV-specific  
15 antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Monospecific polyclonal antibodies to EIV PR/56 NA were shown to react specifically with S-SPV-034 plaques and not with S-SPV-001 negative control plaques. All S-SPV-034 observed plaques reacted with  
20 the antiserum indicating that the virus was stably expressing the EIV PR/56 NA gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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Example 18CS-SPV-038:

30 S-SPV-038 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (*lacZ*) and the gene for equine herpesvirus type 1 glycoprotein B are inserted into the SPV 617-48.1 ORF (a unique *NotI* restriction site has replaced a unique *AccI* restriction  
35 site). The *lacZ* gene is under the control of the synthetic

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late promoter (LP1), and the EHV-1 gB gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-038 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the homology vector 744-34 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-038. This virus is assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

#### Example 18D

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#### S-SPV-039:

S-SPV-039 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for equine herpesvirus type 1 glycoprotein D are inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the EHV-1 gD gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-039 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the homology vector 744-38 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The

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transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS).. The final result of red plaque purification is the recombinant virus designated S-SPV-039. This virus is  
5 assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed  
10 are blue indicating that the virus is pure, stable, and expressing the foreign gene.

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Example 19

Recombinant swinepox viruses express bovine respiratory syncytial virus attachment protein (BRSV G), BRSV Fusion protein (BRSV F), BRSV nucleocapsid protein (BRSV N), bovine viral diarrhea virus (BVDV) gp48, BVDV gp53, bovine parainfluenza virus type 3 (BPI-3) F, or BPI-3 HN. S-SPV-020, S-SPV-029, S-SPV-030, and S-SPV-032, S-SPV-028 are useful as vaccines against bovine disease. These BRSV, BVDV, and BPI-3 antigens are key to raising a protective immune response in the animal. The recombinant viruses are useful alone or in combination as an effective vaccine. The swinepox virus is useful for cloning other subtypes of BRSV, BVDV, and BPI-3 to protect against rapidly evolving variants in this disease. S-SPV-020, S-SPV-029, S-SPV-030, and S-SPV-032, S-SPV-028 are also useful as an expression vector for expressing BRSV, BVDV, and BPI-3 antigens. Such BRSV, BVDV, and BPI-3 antigens are useful to identify antibodies directed against the wild-type BRSV, BVDV, and BPI-3. The viruses are also useful as antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these viruses according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

Example 19A

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S-SPV-020:

S-SPV-020 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for bovine respiratory syncytial virus (BRSV)

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G were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BRSV G gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-020 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-20.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-020. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-SPV-020 was assayed for expression of BRSV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Bovine anti-BRSV FITC (Accurate Chemicals) was shown to react specifically with S-SPV-020 plaques and not with S-SPV-003 negative control plaques. All S-SPV-020 observed plaques reacted with the antiserum indicating that the virus was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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To confirm the expression of the BRSV G gene product, cells were infected with S-SPV-020 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Bovine anti-BRSV FITC (Accurate Chemicals) was used to detect expression of BRSV specific proteins. The lysate from S-SPV-020 infected cells exhibited a band at 36 kd which is the expected size of the non-glycosylated form of BRSV G protein and bands at 43 to 45 kd and 80 to 90 kd which are the expected size of glycosylated forms of the BRSV G protein.

#### Example 19B

##### 15 S-SPV-029:

S-SPV-029 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for bovine respiratory syncytial virus (BRSV) F were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BRSV F gene is under the control of the synthetic late/early promoter (LP2EP2).

25 S-SPV-029 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-20.10 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-029. This virus was assayed for  $\beta$ -galactosidase expression, 35 purity, and insert stability by multiple passages monitored



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by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-SPV-029 was assayed for expression of BRSV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Bovine anti-BRSV FITC (Accurate Chemicals) was shown to react specifically with S-  
10 SPV-029 plaques and not with S-SPV-003 negative control plaques. All S-SPV-029 observed plaques reacted with the antiserum indicating that the virus was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells  
15 would be a suitable substrate for the production of SPV recombinant vaccines.

#### Example 19C

20 S-SPV-030:

S-SPV-030 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (*lacZ*) and the gene for bovine respiratory syncytial virus (BRSV)  
25 N were inserted into the SPV 617-48.1 ORF (a unique *NotI* restriction site has replaced a unique *AccI* restriction site). The *lacZ* gene is under the control of the synthetic late promoter (LP1), and the BRSV N gene is under the control of the synthetic late/early promoter (LP2EP2).

30 S-SPV-030 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 713-55.37 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING  
35 RECOMBINANT SPV. The transfection stock was screened by the

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SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-030. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

10

S-SPV-030 was assayed for expression of BRSV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Bovine anti-BRSV FITC (Accurate Chemicals) was shown to react specifically with

15

h S-SPV-030 plaques and not with S-SPV-003 negative control plaques. All S-SPV-030 observed plaques reacted with the antiserum indicating that the virus was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

20

To confirm the expression of the BRSV N gene product, cells were infected with SPV-030 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Bovine anti-BRSV FITC (Accurate Chemicals) was used to detect expression of BRSV specific proteins. The lysate from S-SPV-030 infected cells exhibited a band at 43 kd which is the expected size of the BRSV N protein.

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#### Example 19D

35 S-SPV-028:

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- S-SPV-028 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for bovine parainfluenza virus type 3 (BPI-3) F were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BPI-3 F gene is under the control of the synthetic late/early promoter (LP2EP2).
- 10 S-SPV-028 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 713-55.10 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the
- 15 SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-028. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored
- 20 by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.
- 25 S-SPV-028 was assayed for expression of BPI-3-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Bovine anti-BPI-3 FITC (Accurate Chemicals) was shown to react specifically with S-SPV-028 plaques and not with S-SPV-003 negative control
- 30 plaques. All S-SPV-028 observed plaques reacted with the antiserum indicating that the virus was stably expressing the BPI-3 foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV
- 35 recombinant vaccines.

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To confirm the expression of the BPI-3 F gene product, cells were infected with SPV-028 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Bovine anti-BPI-3 FITC (Accurate Chemicals) was used to detect expression of BPI-3 specific proteins. The lysate from S-SPV-028 infected cells exhibited bands at 43, and 70 kd which is the expected size of the BPI-3 F protein.

#### Example 19E

##### S-SPV-032:

S-SPV-032 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (*lacZ*) and the gene for bovine viral diarrhea virus (BVDV) gp48 were inserted into the SPV 617-48.1 ORF (a unique *NotI* restriction site has replaced a unique *AccI* restriction site). The *lacZ* gene is under the control of the synthetic late promoter (LP1), and the BVDV gp48 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-032 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-78.1 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-032. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed

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were blue indicating that the virus was pure, stable, and expressing the foreign gene.

Example 19F

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S-SPV-040:

10 S-SPV-040 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for bovine viral diarrhea virus (BVDV) gp53 were inserted into the SPV 617-48.1 ORF (a unique *NotI* restriction site has replaced a unique *AccI* restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the BVDV gp53 gene is under the control of the synthetic late/early promoter (LP2EP2).

20 S-SPV-040 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the homology vector 738-96 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus designated S-SPV-040. This virus is  
25 assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and  
30 expressing the foreign gene.

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Example 19GShipping Fever Vaccine

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Shipping fever or bovine respiratory disease (BRD) complex is manifested as the result of a combination of infectious diseases of cattle and additional stress related factors (52). Respiratory virus infections augmented by pathophysiological effects of stress, alter the susceptibility of cattle to Pasteurella organisms by a number of mechanisms. Control of the viral infections that initiate BRD is essential to preventing the disease syndrome (53).

15

The major infectious disease pathogens that contribute to BRD include but are not limited to infectious bovine rhinotracheitis virus (IBRV), parainfluenza virus type 3 (PI-3), bovine respiratory syncytial virus (BRSV), and Pasteurella haemolytica (53). Recombinant swinepox virus expressing protective antigens to organisms causing BRD is useful as a vaccine. S-SPV-020, S-SPV-029, S-SPV-030, S-SPV-032, and S-SPV-028 are useful components of such a vaccine.

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Example 20

Recombinant swinepox viruses S-SPV-031 and S-SPV-035 are useful as a vaccine against human disease. S-SPV-031 expresses the core antigen of hepatitis B virus. S-SPV-031 is useful against hepatitis B infection in humans. S-SPV-035 expresses the cytokine, interleukin-2, and is useful as an immune modulator to enhance an immune response in humans. When S-SPV-031 and S-SPV-035 are combined, a superior vaccine against hepatitis B is produced.

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Example 20AS-SPV-031:

5 S-SPV-031 is a swinepox virus that expresses at least two  
foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ)  
and the gene for Hepatitis B Core antigen were inserted into  
the SPV 617-48.1 ORF (a unique *NotI* restriction site has  
replaced a unique *AccI* restriction site). The lacZ gene is  
10 under the control of the synthetic late promoter (LP1), and  
the Hepatitis B Core antigen gene is under the control of  
the synthetic early/late promoter (EP1LP2).

S-SPV-031 was derived from S-SPV-001 (Kasza Strain). This  
15 was accomplished utilizing the homology vector 727-67.18  
(see Materials and Methods) and virus S-SPV-001 in the  
HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING  
RECOMBINANT SPV. The transfection stock was screened by the  
SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase  
20 (BLUOGAL AND CPRG ASSAYS). The final result of red plaque  
purification was the recombinant virus designated S-SPV-031.  
This virus was assayed for  $\beta$ -galactosidase expression,  
purity, and insert stability by multiple passages monitored  
by the blue plaque assay as described in Materials and  
25 Methods. After the initial three rounds of purification, all  
plaques observed were blue indicating that the virus was  
pure, stable, and expressing the foreign gene.

30 S-SPV-031 was assayed for expression of Hepatitis B Core  
antigen-specific antigens using the BLACK PLAQUE SCREEN FOR  
FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rabbit antisera  
to Hepatitis B Core antigen was shown to react specifically  
with S-SPV-031 plaques and not with S-SPV-001 negative  
35 control plaques. All S-SPV-031 observed plaques reacted with

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the antiserum indicating that the virus was stably expressing the Hepatitis B Core antigen gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the Hepatitis B Core antigen gene product, cells were infected with SPV-031 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Rabbit antisera to Hepatitis B Core antigen was used to detect expression of Hepatitis B specific proteins. The lysate from S-SPV-031 infected cells exhibited a band at 21 kd which is the expected size of the Hepatitis B Core antigen.

#### Example 20B

##### S-SPV-035:

S-SPV-035 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (*lacZ*) and the gene for human IL-2 were inserted into the SPV 617-48.1 ORF (a unique *NotI* restriction site has replaced a unique *AccI* restriction site). The *lacZ* gene is under the control of the synthetic late promoter (LP1), and the human IL-2 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-SPV-035 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 741-84.14 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase



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(BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-035. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

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Example 21

Human vaccines using recombinant swinepox virus as a vector

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Recombinant swinepox virus is useful as a vaccine against human diseases. For example, human influenza virus is a rapidly evolving virus whose neutralizing viral epitopes rapidly change. A useful recombinant swinepox vaccine is one in which the influenza virus neutralizing epitopes are quickly adapted by recombinant DNA techniques to protect against new strains of influenza virus. Human influenza virus hemagglutinin (HN) and neuraminidase (NA) genes are cloned into the swinepox virus as described in CLONING OF EQUINE INFLUENZA VIRUS HEMAGGLUTININ AND NEURAMINIDASE GENES (See Materials and Methods and Example 17).

Recombinant swinepox virus is useful as a vaccine against other human diseases when foreign antigens from the following diseases or disease organisms are expressed in the swinepox virus vector: hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, human herpesviruses, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus, rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, Diphtheria, *Rickettsia prowazekii*, *Borrelia burgdorferi*, Tetanus toxoid, malignant tumor antigens.

Furthermore, S-SPV-035 (Example 20), when combined with swinepox virus interleukin-2 is useful in enhancing immune

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response in humans. Additional cytokines, including but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, interleukin receptors from human and other animals when vectored into a non-essential site in the swinepox viral genome, and subsequently expressed, have immune stimulating effects.

Recombinant swinepox virus express foreign genes in a human cell line. We demonstrated that S-SPV-003 (EP1LP2 promoter expressing the lacZ gen) expressed the lacZ gene in THP human monocyte cell lines by measuring  $\beta$ -galactosidase activity. We did not observe any cytopathic effect of swinepox virus on the THP human monocyte cells, indicating that recombinant swinepox virus can express foreign genes in a human cell line, but will not productively infect or replicated in the human cell line. We have demonstrated that swinepox virus replicates well in ESK-4 cells (embryonic swine kidney) indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

#### Example 22

Avian vaccines using recombinant swinepox virus as a vector

#### Example 22A

#### S-SPV-026

S-SPV-026 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for infectious bursal disease virus (IBDV) polyprotein were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI

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restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the IBDV polyprotein gene is under the control of the synthetic early/late promoter (EP1LP2).

5

S-SPV-026 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 689-50.4 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-026. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indication that the virus was pure, stable, and expressing the foreign gene.

20

S-SPV-026 was assayed for expression of IBDV polyprotein-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rat antisera to IBDV polyprotein were shown to react specifically with S-SPV-026 plaques and not with S-SPV-001 negative control plaques. All S-SPV-026 observed plaques reacted with the antiserum indicating that the virus was stably expressing the IBDV polyprotein gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

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To confirm the expression of the IBDV polyprotein gene product, cells were infected with SPV-026 and samples of infected cell lysates were subjected to SDS-polyacrylamide

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gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Rat antisera to IBDV proteins VP2, VP3, and VP4 and monoclonal antibody R63 to IBDV VP2 were used to detect expression of IBDV proteins.

5 The lysate from S-SPV-026 infected cells exhibited bands at 32 to 40 kd which is the expected size of the IBDV proteins.

## Example 22B

10 S-SPV-027

S-SPV-027 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) and the gene for infectious bursal disease virus (IBDV) VP2

15 (40kd) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the IBDV VP2 gene is under the control of the synthetic early/late promoter (EP1LP2).

20

S-SPV-027 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 689-50.7 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The

25 transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-027. This virus was assayed for  $\beta$ -galactosidase expression, purity, and insert

30 stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

35

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S-SPV-027 was assayed for expression of IBDV VP2-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rat antisera to IBDV protein was shown to react specifically with S-SPV-027 plaques and not with S-SPV-001 negative control plaques. All S-SPV-027 observed plaques reacted with the antiserum indicating that the virus was stably expressing the IBDV VP2 gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be a suitable substrate for the production of SPV recombinant vaccines.

To confirm the expression of the IBDV VP2 gene product, cells were infected with S-SPV-027 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Rat antisera to IBDV protein and monoclonal antibody R63 to IBDV VP2 were used to detect expression of IBDV VP2 protein. The lysate from S-SPV-027 infected cells exhibited a band at 40 kd which is the expected size of the IBDV VP2 protein.

S-SPV-026 and S-SPV-027 are useful as vaccines against infectious bursal disease in chickens and also as expression vectors for IBDV proteins. Recombinant swinepox virus is useful as a vaccine against other avian disease when foreign antigens from the following diseases or disease organisms are expressed in the swinepox virus vector: Marek's disease virus, infectious laryngotracheitis virus, Newcastle disease virus, infectious bronchitis virus, and chicken anemia virus.

#### Example 23

#### SPV-036:

35

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S-SPV-036 is a swinepox virus that expresses at one foreign gene. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) was inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the human cytomegalovirus immediate early (HCMV IE) promoter.

S-SPV-036 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 741-80.3 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-036. This virus is assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene.

The expression of lacZ from the HCMV IE promoter provides a strong promoter for expression of foreign genes in swinepox. S-SPV-036 is a novel and unexpected demonstration of a herpesvirus promoter driving expression of a foreign gene in a poxvirus. S-SPV-036 is useful in formulating human vaccines, and recombinant swinepox virus is useful for the expression of neutralizing antigens from human pathogens. Recombinant swinepox virus expressed foreign genes in a human cell line as demonstrated by S-SPV-003 (EP1LP2) promoter expressing the lacZ gene) expressed  $\beta$ -galactosidase in THP human monocyte cell lines.

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Recombinant swinepox virus expressed foreign genes in a human cell line as demonstrated by s-SPV-003 (EP1LP2 promoter expressing the lacZ gene) expressed  $\beta$ -galactosidase in THP human monocyte cell lines. THP human monocyte cells are useful for the production of recombinant swinepox virus as a human vaccine. Other cell lines in which swinepox virus will replicate include, but are not limited to, Vero cells (monkey), ST cells (swine testicle), PK-15 (porcine kidney), and ESK-4 cells (embryonic swine kidney).

#### Example 24

##### Homology Vector 738-94.5

Homology Vector 738-94.5 is a swinepox virus vector that expresses one foreign gene. The gene for *E. coli*  $\beta$ -galactosidase (lacZ) was inserted into the the O1L open reading frame (SEQ ID NO. 115). The lacZ gene is under the control of the O1L promoter. The homology vector 738-94.5 contains a deletion of SPV DNA from nucleotides 1381 to 2133 (SEQ ID NO. 113; Figure 17) which deletes part of the O1L ORF.

The upstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-GAAGCATGCCCGTTCTTATCAATAGTTTAGTCGAAAATA-3' (SEQ ID NO. 185) and 5'-CATAAGATCTGGCATTGTGTTATTATACTAACAAAAATAAG-3' (SEQ ID NO. 186) to produce an 871 base pair fragment with BglII and SphI ends. The O1L promoter is present on this fragment. The downstream SPV sequences were synthesized by polymerase chain reaction using DNA primers 5'-CCGTAGTCGACAAAGATCGACTTATTAATATGTATGGGATT-3' (SEQ ID NO. 187) and 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' (SEQ ID NO. 188) to produce an 1123 base pair fragment with SalI and HindIII ends. A recombinant swinepox virus was derived



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utilizing homology vector 738-94.5 and S-SPV-001 (Kasza strain) in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING  $\beta$ -galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant virus. This virus is assayed for  $\beta$ -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and expressing the foreign gene. Recombinant swinepox viruses derived from homology vector 738-94.5 are utilized as an expression vector to express foreign antigens and as a vaccine to raise a protective immune response in animals to foreign genes expressed by the recombinant swinepox virus. Other promoters in addition to the O1L promoter are inserted into the deleted region including LP1, EP1LP2, LP2EP2, HCMV immediate early, and one or more foreign genes are expressed from these promoters.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Cochran Ph.D., Mark D  
Junker M.S., David E
- (ii) TITLE OF INVENTION: Recombinant Swinepox Virus
- (iii) NUMBER OF SEQUENCES: 188
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: John P. White
  - (B) STREET: 30 Rockefeller Plaza
  - (C) CITY: New York
  - (D) STATE: New York
  - (E) COUNTRY: USA
  - (F) ZIP: 10112
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: Not Yet Known
  - (B) FILING DATE: Herewith
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: White, John P
- (ix) TELECOMMUNICATION INFORMATION:
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  - (B) TELEFAX: (212)664-0525
  - (C) TELEX: 422523

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 599 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

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- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Swinepox virus  
 (B) STRAIN: Kasza  
 (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:  
 (B) MAP POSITION: -23.2  
 (C) UNITS: %G
- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 202..597  
 (D) OTHER INFORMATION: /partial  
       /codon\_start= 202  
       /function= "Potential eukaryotic transcriptional  
       regulatory protein"  
       /standard\_name= "515-85.1 ORF"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATGGTTATCA TATACATTTT TAAGGCCGTA TATGATAAAT GAAAATATAT AAGCACTTAT	180
TTTTGTTAGT ATAATAACAC A ATG CCG TCG TAT ATG TAT CCG AAG AAC GCA	231
Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala	
1 5 10	
AGA AAA GTA ATT TCA AAG ATT ATA TCA TTA CAA CTT GAT ATT AAA AAA	279
Arg Lys Val Ile Ser Lys Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys	
15 20 25	
CTT CCT AAA AAA TAT ATA AAT ACC ATG TTA GAA TTT GGT CTA CAT GGA	327
Leu Pro Lys Lys Tyr Ile Asn Thr Met Leu Glu Phe Gly Leu His Gly	
30 35 40	
AAT CTA CCA GCT TGT ATG TAT AAA GAT GCC GTA TCA TAT GAT ATA AAT	375
Asn Leu Pro Ala Cys Met Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn	
45 50 55	
AAT ATA AGA TTT TTA CCT TAT AAT TGT GTT ATG GTT AAA GAT TTA ATA	423
Asn Ile Arg Phe Leu Pro Tyr Asn Cys Val Met Val Lys Asp Leu Ile	
60 65 70	
AAT GTT ATA AAA TCA TCA TCT GTA ATA GAT ACT AGA TTA CAT CAA TCT	471
Asn Val Ile Lys Ser Ser Val Ile Asp Thr Arg Leu His Gln Ser	
75 80 85 90	
GTA TTA AAA CAT CGT AGA GCG TTA ATA GAT TAC GGC GAT CAA GAC ATT	519
Val Leu Lys His Arg Arg Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile	
95 100 105	
ATC ACT TTA ATG ATC ATT AAT AAG TTA CTA TCG ATA GAT GAT ATA TCC	567
Ile Thr Leu Met Ile Ile Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser	
110 115 120	



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TAT ATA TTA GAT AAA AAA ATA ATT CAT GTA AC  
 Tyr Ile Leu Asp Lys Lys Ile Ile His Val  
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599

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys  
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                           20                          25                          30  
 Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met  
                           35                          40                          45  
 Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro  
           50                          55                          60  
 Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser  
   65                          70                          75                          80  
 Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg  
                           85                          90                          95  
 Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile  
                           100                          105                          110  
 Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys  
           115                          120                          125  
 Ile Ile His Val  
   130

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 899 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

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(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 3..662  
(D) OTHER INFORMATION: /partial  
/codon\_start= 3  
/function= "Potential eukaryotic transcriptional  
regulatory protein"  
/standard\_name= "515-85.1 ORP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Asp Ile Lys Ser Cys Lys Cys Ser Ile Cys Ser Asp Ser Ile Thr	
1 5 10 15	
CAT CAT ATA TAT GAA ACA ACA TCA TGT ATA AAT TAT AAA TCT ACC GAT	95
His His Ile Tyr Glu Thr Thr Ser Cys Ile Asn Tyr Lys Ser Thr Asp	
20 25 30	
AAT GAT CTT ATG ATA GTA TTG TTC AAT CTA ACT AGA TAT TTA ATG CAT	143
Asn Asp Leu Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His	
35 40 45	
GGG ATG ATA CAT CCT AAT CTT ATA AGC GTA AAA GGA TGG GGT CCC CTT	191
Gly Met Ile His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu	
50 55 60	
ATT GGA TTA TTA ACG GGT GAT ATA GGT ATT AAT TTA AAA CTA TAT TCC	239
Ile Gly Leu Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser	
65 70 75	
ACC ATG AAT ATA AAT GGG CTA CGG TAT GGA GAT ATT ACG TTA TCT TCA	287
Thr Met Asn Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser	
80 85 90 95	
TAC GAT ATG AGT AAT AAA TTA GTC TCT ATT ATT AAT ACA CCC ATA TAT	335
Tyr Asp Met Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr	
100 105 110	
GAG TTA ATA CCG TTT ACT ACA TGT TGT TCA CTC AAT GAA TAT TAT TCA	383
Glu Leu Ile Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser	
115 120 125	
AAA ATT GTG ATT TTA ATA AAT GTT ATT TTA GAA TAT ATG ATA TCT ATT	431
Lys Ile Val Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile	
130 135 140	
ATA TTA TAT AGA ATA TTG ATC GTA AAA AGA TTT AAT AAC ATT AAA GAA	479
Ile Leu Tyr Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu	
145 150 155	
TTT ATT TCA AAA GTC GTA AAT ACT GTA CTA GAA TCA TCA GGC ATA TAT	527
Phe Ile Ser Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr	
160 165 170 175	

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TTT TGT CAG ATG CGT GTA CAT GAA CAA ATT GAA TTG GAA ATA GAT GAG      575
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CTC ATT ATT AAT GGA TCT ATG CCT GTA CAG CTT ATG CAT TTA CTT CTA      623
Leu Ile Ile Asn Gly Ser Met Pro Val Gln Leu Met His Leu Leu Leu
      195                               200                               205

AAG GTA GCT ACC ATA ATA TTA GAG GAA ATC AAA GAA ATA TAACGTATTT      672
Lys Val Ala Thr Ile Ile Leu Glu Glu Ile Lys Glu Ile
      210                               215                               220

TTTCTTTTAA ATAAATAAAAA ATACTTTTTT TTTTAAACAA GGGGTGCTAC CTTGTCTAAT      732

TGTATCTTGT ATTTTGGATC TGATGCAAGA TTATTAAATA ATCGTATGAA AAAGTAGTAG      792

ATATAGTTTA TATCGTTACT GGACATGATA TTATGTTTAG TTAATCTTTC TTTGGCATGA      852

ATTCTACACG TCGGANAAGG TAATGTATCT ATAATGGTAT AAAGCTT      899

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 220 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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      20                               25                               30

Asp Leu Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His Gly
      35                               40                               45

Met Ile His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu Ile
      50                               55                               60

Gly Leu Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser Thr
      65                               70                               75                               80

Met Asn Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser Tyr
      85                               90                               95

Asp Met Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr Glu
      100                               105                               110

Leu Ile Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser Lys
      115                               120                               125

Ile Val Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile Ile
      130                               135                               140

Leu Tyr Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu Phe
      145                               150                               155                               160

Ile Ser Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr Phe

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	165		170		175										
Cys	Gln	Met	Arg	Val	His	Glu	Gln	Ile	Glu	Leu	Glu	Ile	Asp	Glu	Leu
			180					185					190		
Ile	Ile	Asn	Gly	Ser	Met	Pro	Val	Gln	Leu	Met	His	Leu	Leu	Lys	
		195					200					205			
Val	Ala	Thr	Ile	Ile	Leu	Glu	Glu	Ile	Lys	Glu	Ile				
	210					215					220				

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: YES

## (iv) ANTI-SENSE: NO

## (v) FRAGMENT TYPE: N-terminal

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Vaccinia virus
- (B) STRAIN: Copenhagen

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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			20					25					30		
Leu	Leu	Asp	Tyr	Gly	Leu	His	Gly	Leu	Leu	Pro	Lys	Ser	Leu	Tyr	Leu
		35					40					45			
Glu	Ala	Ile	Asn	Ser	Asp	Ile	Leu	Asn	Val	Arg	Phe	Phe	Pro	Pro	Glu
	50					55					60				
Ile	Ile	Asn	Val	Thr	Asp	Ile	Val	Lys	Ala	Leu	Gln	Asn	Ser	Cys	Arg
65				70					75					80	
Val	Asp	Glu	Tyr	Leu	Lys	Ala	Val	Ser	Leu	Tyr	His	Lys	Asn	Ser	Leu
			85					90					95		
Met	Val	Ser	Gly	Pro	Asn	Val	Val	Lys	Leu	Met	Ile	Glu	Tyr	Asn	Leu
			100					105						110	

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Leu Thr His Ser Asp Leu Glu Trp Leu Ile Asn Glu Asn Val Val Lys  
 115 120 125

Ala

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys  
 1 5 10 15

Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile  
 20 25 30

Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met  
 35 40 45

Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro  
 50 55 60

Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser  
 65 70 75 80

Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg  
 85 90 95

Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile  
 100 105 110

Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys  
 115 120 125

Ile Ile His Val  
 130

## (2) INFORMATION FOR SEQ ID NO:7:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 101 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: C-terminal
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Vaccinia virus
  - (B) STRAIN: Copenhagen
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: ~23.2
  - (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Val Leu Asn Asp Gln Tyr Ala Lys Ile Val Ile Phe Phe Asn Thr Ile
1             5             10             15
Ile Glu Tyr Ile Ile Ala Thr Ile Tyr Tyr Arg Leu Thr Val Leu Asn
20             25             30
Asn Tyr Thr Asn Val Lys His Phe Val Ser Lys Val Leu His Thr Val
35             40             45
Met Glu Ala Cys Gly Val Leu Phe Ser Tyr Ile Lys Val Asn Asp Lys
50             55             60
Ile Glu His Glu Leu Glu Glu Met Val Asp Lys Gly Thr Val Pro Ser
65             70             75             80
Tyr Leu Tyr His Leu Ser Ile Asn Val Ile Ser Ile Ile Leu Asp Asp
85             90             95
Ile Asn Gly Thr Arg
100

```

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 100 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: C-terminal

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- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza

- (viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val Ile  
1 5 10 15  
Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val Lys  
20 25 30  
Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr Val  
35 40 45  
Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu Gln  
50 55 60  
Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro Val  
65 70 75 80  
Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu Glu  
85 90 95  
Ile Lys Glu Ile  
100

- (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 102 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: circular

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:  
(B) CLONE: 520-17.5 (Junction A)

- (x) PUBLICATION INFORMATION:  
(A) AUTHORS: Ferrari, Franco A  
Trach, Kathleen  
Hoch, James A  
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals a  
Polycistronic Transcription Unit  
(C) JOURNAL: J. Bacteriol.  
(D) VOLUME: 161

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(E) ISSUE: 2  
(F) PAGES: 556-562  
(G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATTACC 60  
TTGTCCGACG TGTAGAATTC ATGCCAAAGA AGAATTA ACT AA 102

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)  
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 520-17.5 (Junction B)

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 85..99  
(D) OTHER INFORMATION: /codon\_start= 85  
/function= "Translational start of hybrid protein"  
/product= "N-terminal peptide"  
/number= 1  
/standard\_name= "Translation of synthetic DNA  
sequence"

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 100..102  
(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /partial  
/codon\_start= 100  
/function= "marker enzyme"  
/product= "Beta-Galactosidase"  
/evidence= EXPERIMENTAL  
/gene= "lacZ"  
/number= 2  
/citation= ([1])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A  
Trach, Kathleen  
Hoch, James A  
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals  
a Polycistronic Transcription Unit  
(C) JOURNAL: J. Bacteriol.  
(D) VOLUME: 161  
(E) ISSUE: 2  
(F) PAGES: 556-562



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(G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
GTAGTCGACT CTAGAAAAAA TTGAAAAACT ATTCTAATTT ATTGCACGGA GATCTTTTTT      60
TTTTTTTTTT TTTTGGCAT ATAA ATG AAT TCG GAT CCC GTC      102
                Met Asn Ser Asp Pro Val
                  1                5  1
```

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Asn Ser Asp Pro
 1                5
```

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Val
 1
```

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 103 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 520-17.5 (Junction C)

(ix) FEATURE:

(A) NAME/KEY: CDS

```
(B) LOCATION: 1..72
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /partial
    /codon_start= 1
    /function= "marker enzyme"
    /product= "Beta-galactosidase"
    /evidence= EXPERIMENTAL
    /gene= "lacZ"
    /number= 1
    /citation= {[1]}
```

```
(A) NAME/KEY: CDS
(B) LOCATION: 73..78
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /codon_start= 73
    /function= "Translational finish of hybrid
    protein"
    /product= "C-terminal peptide"
    /evidence= EXPERIMENTAL
    /number= 2
    /standard_name= "Translation of synthetic DNA
    sequence"
```

(A) AUTHORS: Ferrari, Franco A  
Trach, Kathleen  
Hoch, James A  
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals  
a Polycistronic Transcription Unit  
(C) JOURNAL: J. Bacteriol.  
(D) VOLUME: 161  
(E) ISSUE: 2  
(F) PAGES: 556-562  
(G) DATE: Feb.-1985

AGC	CCG	TCA	GTA	TCG	GCG	GAA	ATC	CAG	CTG	AGC	GCC	GGT	CGC	TAC	CAT	48
Ser	Pro	Ser	Val	Ser	Ala	Glu	Ile	Gln	Leu	Ser	Ala	Gly	Arg	Tyr	His	
1				5					10					15		
TAC	CAG	TTG	GTC	TGG	TGT	CAA	AAA	GAT	CCA	TAATTAATTA	ACCCGGGTCG	98				
Tyr	Gln	Leu	Val	Trp	Cys	Gln	Lys	Asp	Pro							
			20					1								
AAGAC											103					

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO:14:

Ser Pro Ser Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His  
1 5 10 15

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Tyr Gln Leu Val Trp Cys Gln Lys  
20

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Pro  
1

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 48 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 520-17.5 (Junction D)

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGGTCATAG CTGTTTCC

48

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:

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(B) CLONE: 538-46.26 (Junction A)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATT

57

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.16 (Junction B)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 91..102
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /partial
  - /codon\_start= 91
  - /function= "marker enzyme"
  - /product= "Beta-Galactosidase"
  - /evidence= EXPERIMENTAL
  - /gene= "lacZ"
  - /number= 2
  - /citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 76..90
- (D) OTHER INFORMATION: /partial
  - /codon\_start= 76
  - /function= "Translational start of hybrid protein"
  - /product= "N-terminal peptide"
  - /number= 1
  - /standard\_name= "Translation of synthetic DNA sequence"

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Ferrari, Franco A  
Trach, Kathleen  
Hoch, James A
- (B) TITLE: Sequence Analysis of the spo0B Locus Reveals  
a Polycistronic Transcription Unit
- (C) JOURNAL: J. Bacteriol.
- (D) VOLUME: 161
- (E) ISSUE: 2
- (F) PAGES: 556-562

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(G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
AAGCTGGTAG ATTTCCATGT AGGGCCGCCT GCAGGTCGAC TCTAGAATTT CATTTTGTTT      60
TTTTCTATGC TATAA ATG AAT TCG GAT CCC GTC GTT TTA CAA      102
           Met Asn Ser Asp Pro Val Val Leu Gln
                1             5 1
```

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Met Asn Ser Asp Pro
 1             5
```

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 4 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Val Val Leu Gln
 1
```

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 206 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.16 (Junction C)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..63

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(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /partial  
    /codon\_start= 1  
    /function= "marker enzyme"  
    /product= "Beta-galactosidase"  
    /evidence= EXPERIMENTAL  
    /number= 1  
    /citation= ([1])

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 64..69  
(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /codon\_start= 64  
    /function= "Translational finish of hybrid  
    protein"  
    /product= "C-terminal peptide"  
    /evidence= EXPERIMENTAL  
    /standard\_name= "Translation of synthetic DNA  
    sequence"

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 177..185  
(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /codon\_start= 177  
    /function= "Translational start of hybrid protein"  
    /product= "N-terminal peptide"  
    /evidence= EXPERIMENTAL  
    /standard\_name= "Translation of synthetic DNA  
    sequence"

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 186..206  
(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /partial  
    /codon\_start= 186  
    /function= "glycoprotein"  
    /product= "PRV gp50"  
    /evidence= EXPERIMENTAL  
    /gene= "gp50"  
    /number= 3  
    /citation= ([2])

## (x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A  
            Trach, Kathleen  
            Hoch, James A  
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals  
          a Polycistronic Transcription Unit  
(C) JOURNAL: J. Bacteriol.  
(D) VOLUME: 161  
(E) ISSUE: 2  
(F) PAGES: 556-562  
(G) DATE: Feb.-1985

## (x) PUBLICATION INFORMATION:

(A) AUTHORS: Petrovskis, Erik A  
            Timmins, James G  
            Armentrout, Marty A  
            Marchioli, Carmine C  
            Jr. Yancy, Robert J

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Post, Leonard E

- (B) TITLE: DNA Sequence of the Gene for Pseudorabies  
Virus gp50, a Glycoprotein without N-Linked  
Glycosylation  
(C) JOURNAL: J. Virol.  
(D) VOLUME: 59  
(E) ISSUE: 2  
(F) PAGES: 216-223  
(G) DATE: Aug.-1986

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

GTA TCG GCG GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG      48
Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu
  1             5             10             15

GTC TGG TGT CAA AAA GAT CCA TAATTAATTA ACCCGGCCCGC CTGCAGGTCTG      99
Val Trp Cys Gln Lys Asp Pro
                20             1

ACTCTAGAAA AAATTGAAAA ACTATTCTAA TTTATTGCAC GGAGATCTTT TTTTTTTTTT    159

TTTTTTTTTG CATATAA ATG AAT TCG CTC GCA GCG CTA TTG GCG GCG      206
Met Asn Ser Leu Ala Ala Leu Leu Ala Ala
  1             1             5

```

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu
  1             5             10             15

Val Trp Cys Gln Lys
                20

```

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```

Asp Pro
  1

```

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 3 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Asn Ser  
1

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Ala Ala Leu Leu Ala Ala  
1 5

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 101 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.16 (Junction D)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..15
- (D) OTHER INFORMATION: /partial
  - /codon\_start= 1
  - /function= "glycoprotein"
  - /product= "PRV gp63"
  - /gene= "gp63"
  - /number= 1
  - /citation= ([1])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Petrovskis, Erik A  
Timmins, James G  
Post, Lenoard E

(B) TITLE: Use of Lambda-gt11 To Isolate Genes for two



-156-

Pseudorabies Virus Glycoproteins with homology to  
Herpes Simplex Virus and Varicella-Zoster Virus  
Glycoproteins

(C) JOURNAL: J. Virol.  
(D) VOLUME: 60  
(E) ISSUE: 1  
(F) PAGES: 185-193  
(G) DATE: Oct.-1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGC GTG CAC CAC GAG GGA CTCTAGA GGATCCATAA TTAATTAATT AATTTTATC 55  
Arg Val His His Glu  
1 5  
CCGGGTCGAC CTGCAGGCGG CCGGGTCGAC CTGCAGGCGG CCAGAC 101

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Arg Val His His Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 538-46.16 (Junction E)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAA 57

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1907 base pairs  
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Newcastle disease virus
  - (B) STRAIN: B1
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 137-23.803 (PSY1142)
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -50%
  - (C) UNITS: %G
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 92..1822
  - (D) OTHER INFORMATION: /codon\_start= 92  
/product= "NDV heamagglutinin-Neuraminidase"  
/gene= "HN"  
/number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACGGGTAGAA CGGTAAGAGA GGCCGCCCT CAATTGCGAG CCAGACTTCA CAACCTCCGT	60
TCTACCGCTT CACCGACAAC AGTCCTCAAT C ATG GAC CGC GCC GTT AGC CAA	112
Met Asp Arg Ala Val Ser Gln	
1 5	
GTT GCG TTA GAG AAT GAT GAA AGA GAG GCA AAA AAT ACA TGG CGC TTG	160
Val Ala Leu Glu Asn Asp Glu Arg Glu Ala Lys Asn Thr Trp Arg Leu	
10 15 20	
ATA TTC CGG ATT GCA ATC TTA TTC TTA ACA GTA GTG ACC TTG GCT ATA	208
Ile Phe Arg Ile Ala Ile Leu Phe Leu Thr Val Val Thr Leu Ala Ile	
25 30 35	
TCT GTA GCC TCC CTT TTA TAT AGC ATG GGG GCT AGC ACA CCT AGC GAT	256
Ser Val Ala Ser Leu Leu Tyr Ser Met Gly Ala Ser Thr Pro Ser Asp	
40 45 50 55	
CTT GTA GGC ATA CCG ACT AGG ATT TCC AGG GCA GAA GAA AAG ATT ACA	304
Leu Val Gly Ile Pro Thr Arg Ile Ser Arg Ala Glu Glu Lys Ile Thr	
60 65 70	
TCT ACA CTT GGT TCC AAT CAA GAT GTA GTA GAT AGG ATA TAT AAG CAA	352
Ser Thr Leu Gly Ser Asn Gln Asp Val Val Asp Arg Ile Tyr Lys Gln	
75 80 85	
GTG GCC CTT GAG TCT CCA TTG GCA TTG TTA AAT ACT GAG ACC ACA ATT	400
Val Ala Leu Glu Ser Pro Leu Ala Leu Leu Asn Thr Glu Thr Thr Ile	
90 95 100	
ATG AAC GCA ATA ACA TCT CTC TCT TAT CAG ATT AAT GGA GCT GCA AAC	448

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Met	Asn	Ala	Ile	Thr	Ser	Leu	Ser	Tyr	Gln	Ile	Asn	Gly	Ala	Ala	Asn		
105						110					115						
AAC	AGC	GGG	TGG	GGG	GCA	CCT	ATT	CAT	GAC	CCA	GAT	TAT	ATA	GGG	GGG	496	
Asn	Ser	Gly	Trp	Gly	Ala	Pro	Ile	His	Asp	Pro	Asp	Tyr	Ile	Gly	Gly		
120					125					130					135		
ATA	GGC	AAA	GAA	CTC	ATT	GTA	GAT	GAT	GCT	AGT	GAT	GTC	ACA	TCA	TTC	544	
Ile	Gly	Lys	Glu	Leu	Ile	Val	Asp	Asp	Ala	Ser	Asp	Val	Thr	Ser	Phe		
				140					145					150			
TAT	CCC	TCT	GCA	TTT	CAA	GAA	CAT	CTG	AAT	TTT	ATC	CCG	GCG	CCT	ACT	592	
Tyr	Pro	Ser	Ala	Phe	Gln	Glu	His	Leu	Asn	Phe	Ile	Pro	Ala	Pro	Thr		
			155					160					165				
ACA	GGA	TCA	GGT	TGC	ACT	CGA	ATA	CCC	TCA	TTT	GAC	ATG	AGT	GCT	ACC	640	
Thr	Gly	Ser	Gly	Cys	Thr	Arg	Ile	Pro	Ser	Phe	Asp	Met	Ser	Ala	Thr		
		170					175					180					
CAT	TAC	TGC	TAC	ACC	CAT	AAT	GTA	ATA	TTG	TCT	GGA	TGC	AGA	GAT	CAC	688	
His	Tyr	Cys	Tyr	Thr	His	Asn	Val	Ile	Leu	Ser	Gly	Cys	Arg	Asp	His		
	185					190					195						
TCA	CAC	TCA	CAT	CAG	TAT	TTA	GCA	CTT	GGT	GTG	CTC	CGG	ACA	TCT	GCA	736	
Ser	His	Ser	His	Gln	Tyr	Leu	Ala	Leu	Gly	Val	Leu	Arg	Thr	Ser	Ala		
200					205					210					215		
ACA	GGG	AGG	GTA	TTC	TTT	TCT	ACT	CTG	CGT	TCC	ATC	AAC	CTG	GAC	GAC	784	
Thr	Gly	Arg	Val	Phe	Phe	Ser	Thr	Leu	Arg	Ser	Ile	Asn	Leu	Asp	Asp		
			220						225					230			
ACC	CAA	AAT	CGG	AAG	TCT	TGC	AGT	GTG	AGT	GCA	ACT	CCC	CTG	GGT	TGT	832	
Thr	Gln	Asn	Arg	Lys	Ser	Cys	Ser	Val	Ser	Ala	Thr	Pro	Leu	Gly	Cys		
			235					240					245				
GAT	ATG	CTG	TGC	TCG	AAA	GCC	ACG	GAG	ACA	GAG	GAA	GAA	GAT	TAT	AAC	880	
Asp	Met	Leu	Cys	Ser	Lys	Ala	Thr	Glu	Thr	Glu	Glu	Glu	Asp	Tyr	Asn		
		250				255					260						
TCA	GCT	GTC	CCT	ACG	CGG	ATG	GTA	CAT	GGG	AGG	TTA	GGG	TTC	GAC	GGC	928	
Ser	Ala	Val	Pro	Thr	Arg	Met	Val	His	Gly	Arg	Leu	Gly	Phe	Asp	Gly		
	265					270					275						
CAA	TAT	CAC	GAA	AAG	GAC	CTA	GAT	GTC	ACA	ACA	TTA	TTC	GGG	GAC	TGG	976	
Gln	Tyr	His	Glu	Lys	Asp	Leu	Asp	Val	Thr	Thr	Leu	Phe	Gly	Asp	Trp		
280					285				290						295		
GTG	GCC	AAC	TAC	CCA	GGA	GTA	GGG	GGT	GGA	TCT	TTT	ATT	GAC	AGC	CGC	1024	
Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly	Gly	Ser	Phe	Ile	Asp	Ser	Arg		
				300					305					310			
GTG	TGG	TTC	TCA	GTC	TAC	GGA	GGG	TTA	AAA	CCC	AAT	ACA	CCC	AGT	GAC	1072	
Val	Trp	Phe	Ser	Val	Tyr	Gly	Gly	Leu	Lys	Pro	Asn	Thr	Pro	Ser	Asp		
			315					320					325				
ACT	GTA	CAG	GAA	GGG	AAA	TAT	GTG	ATA	TAC	AAG	CGA	TAC	AAT	GAC	ACA	1120	
Thr	Val	Gln	Glu	Gly	Lys	Tyr	Val	Ile	Tyr	Lys	Arg	Tyr	Asn	Asp	Thr		
		330					335					340					
TGC	CCA	GAT	GAG	CAA	GAC	TAC	CAG	ATT	CGA	ATG	GCC	AAG	TCT	TCG	TAT	1168	
Cys	Pro	Asp	Glu	Gln	Asp	Tyr	Gln	Ile	Arg	Met	Ala	Lys	Ser	Ser	Tyr		
		345				350					355						

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AAG CCT GGA CGG TTT GGT GGG AAA CGC ATA CAG CAG GCT ATC TTA TCT Lys Pro Gly Arg Phe Gly Gly Lys Arg Ile Gln Gln Ala Ile Leu Ser 360 365 370 375	1216
ATC AAA GTG TCA ACA TCC TTA GGC GAA GAC CCG GTA CTG ACT GTA CCG Ile Lys Val Ser Thr Ser Leu Gly Glu Asp Pro Val Leu Thr Val Pro 380 385 390	1264
CCC AAC ACA GTC ACA CTC ATG GGG GCC GAA GGC AGA ATT CTC ACA GTA Pro Asn Thr Val Thr Leu Met Gly Ala Glu Gly Arg Ile Leu Thr Val 395 400 405	1312
GGG ACA TCC CAT TTC TTG TAT CAG CGA GGG TCA TCA TAC TTC TCT CCC Gly Thr Ser His Phe Leu Tyr Gln Arg Gly Ser Ser Tyr Phe Ser Pro 410 415 420	1360
GCG TTA TTA TAT CCT ATG ACA GTC AGC AAC AAA ACA GCC ACT CTT CAT Ala Leu Leu Tyr Pro Met Thr Val Ser Asn Lys Thr Ala Thr Leu His 425 430 435	1408
AGT CCT TAT ACA TTC AAT GCC TTC ACT CGG CCA GGT AGT ATC CCT TGC Ser Pro Tyr Thr Phe Asn Ala Phe Thr Arg Pro Gly Ser Ile Pro Cys 440 445 450 455	1456
CAG GCT TCA GCA AGA TGC CCC AAC TCA TGT GTT ACT GGA GTC TAT ACA Gln Ala Ser Ala Arg Cys Pro Asn Ser Cys Val Thr Gly Val Tyr Thr 460 465 470	1504
GAT CCA TAT CCC CTA ATC TTC TAT AGA AAC CAC ACC TTG CGA GGG GTA Asp Pro Tyr Pro Leu Ile Phe Tyr Arg Asn His Thr Leu Arg Gly Val 475 480 485	1552
TTC GGG ACA ATG CTT GAT GGT GAA CAA GCA AGA CTT AAC CCT GCG TCT Phe Gly Thr Met Leu Asp Gly Glu Gln Ala Arg Leu Asn Pro Ala Ser 490 495 500	1600
GCA GTA TTC GAT AGC ACA TCC CGC AGT CGC ATA ACT CGA GTG AGT TCA Ala Val Phe Asp Ser Thr Ser Arg Ser Arg Ile Thr Arg Val Ser Ser 505 510 515	1648
AGC AGC ATC AAA GCA GCA TAC ACA ACA TCA ACT TGT TTT AAA GTG GTC Ser Ser Ile Lys Ala Ala Tyr Thr Thr Ser Thr Cys Phe Lys Val Val 520 525 530 535	1696
AAG ACC AAT AAG ACC TAT TGT CTC AGC ATT GCT GAA ATA TCT AAT ACT Lys Thr Asn Lys Thr Tyr Cys Leu Ser Ile Ala Glu Ile Ser Asn Thr 540 545 550	1744
CTC TTC GGA GAA TTC AGA ATC GTC CCG TTA CTA GTT GAG ATC CTC AAA Leu Phe Gly Glu Phe Arg Ile Val Pro Leu Leu Val Glu Ile Leu Lys 555 560 565	1792
GAT GAC GGG GTT AGA GAA GCC AGG TCT GGC TAGTTGAGTC AACTATGAAA Asp Asp Gly Val Arg Glu Ala Arg Ser Gly 570 575	1842
GAGTTGAAAA GATGGCATTG TATCACCTAT CTTCTGCGAC ATCAAGAATC AAACCGAATG CCGGC	1902 1907

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## (2) INFORMATION FOR SEQ ID NO:30:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 577 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

Met Asp Arg Ala Val Ser Gln Val Ala Leu Glu Asn Asp Glu Arg Glu
 1           5           10           15
Ala Lys Asn Thr Trp Arg Leu Ile Phe Arg Ile Ala Ile Leu Phe Leu
          20           25           30
Thr Val Val Thr Leu Ala Ile Ser Val Ala Ser Leu Leu Tyr Ser Met
          35           40           45
Gly Ala Ser Thr Pro Ser Asp Leu Val Gly Ile Pro Thr Arg Ile Ser
          50           55           60
Arg Ala Glu Glu Lys Ile Thr Ser Thr Leu Gly Ser Asn Gln Asp Val
          65           70           75           80
Val Asp Arg Ile Tyr Lys Gln Val Ala Leu Glu Ser Pro Leu Ala Leu
          85           90           95
Leu Asn Thr Glu Thr Thr Ile Met Asn Ala Ile Thr Ser Leu Ser Tyr
          100          105          110
Gln Ile Asn Gly Ala Ala Asn Asn Ser Gly Trp Gly Ala Pro Ile His
          115          120          125
Asp Pro Asp Tyr Ile Gly Gly Ile Gly Lys Glu Leu Ile Val Asp Asp
          130          135          140
Ala Ser Asp Val Thr Ser Phe Tyr Pro Ser Ala Phe Gln Glu His Leu
          145          150          155          160
Asn Phe Ile Pro Ala Pro Thr Thr Gly Ser Gly Cys Thr Arg Ile Pro
          165          170          175
Ser Phe Asp Met Ser Ala Thr His Tyr Cys Tyr Thr His Asn Val Ile
          180          185          190
Leu Ser Gly Cys Arg Asp His Ser His Ser His Gln Tyr Leu Ala Leu
          195          200          205
Gly Val Leu Arg Thr Ser Ala Thr Gly Arg Val Phe Phe Ser Thr Leu
          210          215          220
Arg Ser Ile Asn Leu Asp Asp Thr Gln Asn Arg Lys Ser Cys Ser Val
          225          230          235          240
Ser Ala Thr Pro Leu Gly Cys Asp Met Leu Cys Ser Lys Ala Thr Glu
          245          250          255
Thr Glu Glu Glu Asp Tyr Asn Ser Ala Val Pro Thr Arg Met Val His
          260          265          270

```

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Gly Arg Leu Gly Phe Asp Gly Gln Tyr His Glu Lys Asp Leu Asp Val  
 275 280 285  
 Thr Thr Leu Phe Gly Asp Trp Val Ala Asn Tyr Pro Gly Val Gly Gly  
 290 295 300  
 Gly Ser Phe Ile Asp Ser Arg Val Trp Phe Ser Val Tyr Gly Gly Leu  
 305 310 315 320  
 Lys Pro Asn Thr Pro Ser Asp Thr Val Gln Glu Gly Lys Tyr Val Ile  
 325 330 335  
 Tyr Lys Arg Tyr Asn Asp Thr Cys Pro Asp Glu Gln Asp Tyr Gln Ile  
 340 345 350  
 Arg Met Ala Lys Ser Ser Tyr Lys Pro Gly Arg Phe Gly Gly Lys Arg  
 355 360 365  
 Ile Gln Gln Ala Ile Leu Ser Ile Lys Val Ser Thr Ser Leu Gly Glu  
 370 375 380  
 Asp Pro Val Leu Thr Val Pro Pro Asn Thr Val Thr Leu Met Gly Ala  
 385 390 395 400  
 Glu Gly Arg Ile Leu Thr Val Gly Thr Ser His Phe Leu Tyr Gln Arg  
 405 410 415  
 Gly Ser Ser Tyr Phe Ser Pro Ala Leu Leu Tyr Pro Met Thr Val Ser  
 420 425 430  
 Asn Lys Thr Ala Thr Leu His Ser Pro Tyr Thr Phe Asn Ala Phe Thr  
 435 440 445  
 Arg Pro Gly Ser Ile Pro Cys Gln Ala Ser Ala Arg Cys Pro Asn Ser  
 450 455 460  
 Cys Val Thr Gly Val Tyr Thr Asp Pro Tyr Pro Leu Ile Phe Tyr Arg  
 465 470 475 480  
 Asn His Thr Leu Arg Gly Val Phe Gly Thr Met Leu Asp Gly Glu Gln  
 485 490 495  
 Ala Arg Leu Asn Pro Ala Ser Ala Val Phe Asp Ser Thr Ser Arg Ser  
 500 505 510  
 Arg Ile Thr Arg Val Ser Ser Ser Ser Ile Lys Ala Ala Tyr Thr Thr  
 515 520 525  
 Ser Thr Cys Phe Lys Val Val Lys Thr Asn Lys Thr Tyr Cys Leu Ser  
 530 535 540  
 Ile Ala Glu Ile Ser Asn Thr Leu Phe Gly Glu Phe Arg Ile Val Pro  
 545 550 555 560  
 Leu Leu Val Glu Ile Leu Lys Asp Asp Gly Val Arg Glu Ala Arg Ser  
 565 570 575  
 Gly

(2) INFORMATION FOR SEQ ID NO:31:

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- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Plasmid
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 538-46.26 (Junction A)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATT

57

## (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 108 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Plasmid
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 538-46.26 (Junction B)
- (ix) FEATURE:
  - (A) NAME/KEY: exon
  - (B) LOCATION: 88..102
  - (D) OTHER INFORMATION: /codon\_start= 88  
/function= "Translational start of hybrid protein"  
/product= "N-terminal peptide"  
/number= 1  
/standard\_name= "Translation of synthetic DNA  
sequence"
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 103..108
  - (C) IDENTIFICATION METHOD: experimental
  - (D) OTHER INFORMATION: /partial  
/codon\_start= 103  
/product= "NDV Heamagglutinin-Neuraminidase"  
/evidence= EXPERIMENTAL

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/gene= "HN"  
/number= 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
CATGTAGTCG ACTCTAGAAA AAATTGAAAA ACTATTCTAA TTTATTGCAC GGAGATCTTT      60
TTTTTTTTTT TTTTTTTTGG CATATAAATG AATTCGGATC CG GAC CGC      108
                               Asp Arg
                               1
```

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Asp Arg  
1

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 108 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: 538-46.26 (Junction C)

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 70..84  
 (D) OTHER INFORMATION: /codon\_start= 70  
 /function= "Translational start of hybrid protein"  
 /product= "N-terminal peptide"  
 /number= 1  
 /standard\_name= "Translation of synthetic DNA  
 sequence"

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 85..108



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(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /partial  
    /codon\_start= 85  
    /function= "marker enzyme"  
    /product= "Beta-galactosidase"  
    /evidence= EXPERIMENTAL  
    /gene= "lacZ"  
    /number= 2  
    /citation= ([1])

## (x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A  
            Trach, Kathleen  
            Hoch, James A  
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals  
          a Polycistronic Transcription Unit  
(C) JOURNAL: J. Bacteriol.  
(D) VOLUME: 161  
(E) ISSUE: 2  
(F) PAGES: 556-562  
(G) DATE: Feb.-1985

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
TGCGACATCA AGAATCAAAC CGAATGCCCT CGACTCTAGA ATTTCATTTT GTTTTTTCT      60
ATGCTATAAA ATG AAT TCG GAT CCC GTC GTT TTA CAA CGT CGT GAC TGG      108
Met Asn Ser Asp Pro Val Val Leu Gln Arg Arg Asp Trp
   1             5   1             5
```

## (2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 5 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Met Asn Ser Asp Pro
   1             5
```

## (2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 8 amino acids  
    (B) TYPE: amino acid  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Val Val Leu Gln Arg Arg Asp Trp
   1             5
```

## (2) INFORMATION FOR SEQ ID NO:37:

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- ```

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 108 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:
    (B) CLONE: 538-46.26

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 1..54
    (C) IDENTIFICATION METHOD: experimental
    (D) OTHER INFORMATION: /partial
        /codon_start= 1
        /function= "marker enzyme"
        /product= "Beta-galactosidase"
        /evidence= EXPERIMENTAL
        /gene= "lacZ"
        /number= 1
        /citation= ([1])

(ix) FEATURE:
    (A) NAME/KEY: CDS
    (B) LOCATION: 55..63
    (C) IDENTIFICATION METHOD: experimental
    (D) OTHER INFORMATION: /codon_start= 55
        /function= "Translational finish of hybrid
        protein"
        /product= "C-terminal peptide"
        /evidence= EXPERIMENTAL
        /number= 2
        /standard_name= "Translation of synthetic DNA
        sequence"

```

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT | 48  |
| Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys |     |
| 1 5 10 15                                                       |     |
| CAA AAA GAT CCA TAATTAATTA ACCCGGGTCG AGGGTCGAAG ACCAAATTCT     | 100 |
| Gln Lys Asp Pro                                                 |     |
| 1                                                               |     |
| AACATGGT                                                        | 108 |

- (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
1 5 10 15

Gln Lys

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Pro  
1

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 57 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 538-46.26 (Junction E)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:  
AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAA

57

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Pseudorabies virus \ Synthetic oligonucleotide primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CGCGAATTCTG CTCGCAGCGC TATTGGC

27

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Pseudorabies virus \ Synthetic oligonucleotide primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GTAGGAGTGG CTGCTGAAG

19

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

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(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: ~23.2

(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AAAAATTGAA    AAACTATTCT    AATTTATTGC    ACGGAGATCT    TTTTTTTTTT  
TTTTTTTTTG    60

GCATATAAAT

70

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: ~23.2

(C) UNITS: %G

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TTTTTTTTTT TTTTTTTTTT GGCATATAAA TAGATCTGTA TCCTAAAATT GAATTGTAAT  
60  
TATCGATAAT AAAT 74

## (2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 37 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTATCCTAAA ATTGAATTGT AATTATCGAT AATAAAT

37

## (2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 41 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:

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(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2

(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CGACTCTAGA ATTTCAATTT GTTTTTTCT ATGCTATAAA T

41

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2

(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTGA GTCTATTGGT TATTTATACG

60

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

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(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2

(C) UNITS: %G

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 100..123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
TGAATATATA GCAAATAAAG GAAAAATTGT TATCGTTGCT GCATTAGATG GAACATAGGT      60
CGACTCTAGA ATTCATTTT GTTTTTTCT ATGCTATAA ATG AAT TCG GAT CCC      114
                               Met Asn Ser Asp Pro
                               1           5

GTC GTT TTA      123
Val Val Leu
```

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

```
Met Asn Ser Asp Pro Val Val Leu
1           5
```

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1



(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT | 48  |
| Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys |     |
| 1 5 10 15                                                       |     |
| CAA AAA GAT CCA TAATTAATTA ACCCGGGTCG ACCTATGAAC GTAAACCATT     | 100 |
| Gln Lys Asp Pro                                                 |     |
| 20                                                              |     |
| TGGTAATATT CTTAATCTTA TACCATTATC GG                             | 132 |

(2) INFORMATION FOR SEO ID NO:51:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
1 5 10 15  
Gln Lys Asp Pro  
20

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 66 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Swinepox virus  
 (B) STRAIN: Kasza  
 (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

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(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TCTACTATTG TATATATAGG ATCCCCGGGC GAGCTCGAAT TCGTAATCAT GGTCATAGCT 60  
GTTTCC 66

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 104 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

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(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 81..104

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
AAATATATAA ATACCATGTT AGAATTTGGT CTGCTGCAGG TCGACTCTAG AATTTTCATTT      60
TGTTTTTTTC TATGCTATAA ATG AAT TCG GAT CCC GTC GTT TTA      104
           Met Asn Ser Asp Pro Val Val Leu
             1                   5
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Met Asn Ser Asp Pro Val Val Leu
  1                   5
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 150 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(ix) FEATURE:

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(A) NAME/KEY: CDS  
(B) LOCATION: 1..63

## (ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 130..150

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```

GAA ATC CAG CTG AGC GCC GGT CGC TAC TAC CAG TTG GTC TGG TGT      48
Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys
 1             5             10             15

CAA AAA GAT CCA TAATTAATTA ACCCGGTCGA CTCTAGAAAG ATCTGTATCC      100
Gln Lys Asp Pro
                20

TAAAATTGAA TTGTAATTAT CGATAATAA ATG AAT TCC GGC ATG GCC TCG      150
                Met Asn Ser Gly Met Ala Ser
                1             5

```

## (2) INFORMATION FOR SEQ ID NO:57:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys
 1             5             10             15
Gln Lys Asp Pro
                20

```

## (2) INFORMATION FOR SEQ ID NO:58:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```

Met Asn Ser Gly Met Ala Ser
 1             5

```

## (2) INFORMATION FOR SEQ ID NO:59:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 109 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double

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- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
    (A) ORGANISM: Swinepox virus  
    (B) STRAIN: Kasza  
    (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:  
    (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:  
    (B) MAP POSITION: -23.2  
    (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
CCATGCTCTA GAGGATCCCC GGGCGAGCTC GAATTCGGAT CCATAATTAA TTAATTAATT      60
TTTATCCCGG GTCGACCGGG TCGACCTGCA GCCTACATGG AAATCTACC      109
```

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 51 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: double  
    (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
    (A) ORGANISM: Swinepox virus  
    (B) STRAIN: Kasza  
    (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:  
    (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:  
    (B) MAP POSITION: -23.2  
    (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C      51
```

(2) INFORMATION FOR SEQ ID NO:61:

-177-

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

## (2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 104 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 81..104

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

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AAATATATAA ATACCATGTT AGAATTTGGT CTGCTGCAGG TCGACTCTAG AATTCATTT 60  
 TGTITTTTTTC TATGCTATAA ATG AAT TCG GAT CCC GTC GTT TTA 104  
 Met Asn Ser Asp Pro Val Val Leu  
 1 5

## (2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Met Asn Ser Asp Pro Val Val Leu  
 1 5

## (2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 182 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001

- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1

- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..63

- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 156..182

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT 48  
 Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
 1 5 10 15

|                                                                  |     |
|------------------------------------------------------------------|-----|
| CAA AAA GAT CCA TAATTAATTA ACCCGGTCGA CTCTAGAAAA AATTGAAAAA      | 100 |
| Gln Lys Asp Pro                                                  |     |
| 20                                                               |     |
| CTATTCTAAT TTATTGCACG GAGATCTTTT TTTTTTTTTT TTTTGTGGCA TATAA ATG | 158 |
| Met                                                              |     |
| 1                                                                |     |
| AAT TCC GGC ATG GCC TCG CTC GCG                                  | 182 |
| Asn Ser Gly Met Ala Ser Leu Ala                                  |     |
| 5                                                                |     |

(A) LENGTH: 20 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
1 5 10 15  
Gln Lys Asp Pro  
20

(A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

Met Asn Ser Gly Met Ala Ser Leu Ala  
1 5

(A) LENGTH: 109 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(A) ORGANISM: Swinepox virus



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(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CCATGCTCTA GAGGATCCCC GGGCGAGCTC GAATTCGGAT CCATAATTAA TTAATTAATT 60  
TTTATCCCGG GTCGACCGGG TCGACCTGCA GCCTACATGG AAATCTACC 109

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 81..104

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

AAATATATAA ATACCATGTT AGAATTTGGT CTGCTGCAGG TCGACTCTAG AATTTTCATT

60

TGTTTTTTTC TATGCTATAA ATG AAT TCG GAT CCC GTC GTT TTA

104

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

-182-

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Met Asn Ser Asp Pro Val Val Leu  
 1 5

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 180 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: ~23.2
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..63

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 160..180

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT   | 48  |
| Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys   |     |
| 1 5 10 15                                                         |     |
| CAA AAA GAT CCA TAATTAATTA ACCCGGTCGA CTCTAGATT TTTTTTTTTT        | 100 |
| Gln Lys Asp Pro                                                   |     |
| 20                                                                |     |
| TTTTTTTGGC ATATAAATAG ATCTGTATCC TAA AATTGAA TTGTAATTAT CGATAATAA | 159 |
| ATG AAT TCC GGC ATG GCC TCG                                       | 180 |
| Met Asn Ser Gly Met Ala Ser                                       |     |
| 1 5                                                               |     |

(2) INFORMATION FOR SEQ ID NO:73:

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Gln Lys Asp Pro  
20

(2) INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Met Asn Ser Gly Met Ala Ser  
1 5

(2) INFORMATION FOR SEQ ID NO:75:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 109 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

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CCATGCTCTA GAGGATCCCC GGGCGAGCTC GAATTCGGAT CCATAATTAA TTAATTAATT 60  
TTTATCCCGG GTCGACCGGG TCGACCTGCA GCCTACATGG AAATCTACC 109

## (2) INFORMATION FOR SEQ ID NO:76:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

## (2) INFORMATION FOR SEQ ID NO:77:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 117 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 94..117

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GGTCTGCTGC AGGTCGACTC TAGAAAAAAT TGAAAAACTA TTCTAATTTA TTGCACGGAG

60

ATCTTTTTTT TTTTTTTTTT TTTTGGCATA TAA ATG AAT TCC GGC TTC AGT AAC ATA  
 Met Asn Ser Gly Phe Ser Asn Ile  
 1 5 8

117

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Met Asn Ser Gly Phe Ser Asn Ile  
 1 5

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

-186-

- (A) LENGTH: 126 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 103..126

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```

CGCAACATAC CTAAGTCTT CATTCTGAT CCATAATTAA TTAATTTTAA TCCCGGCGCG      60
CCTCGACTCT AGAATTCAT TTTGTTTTT TCTATGCTAT AA ATG AAT TCG GAT      114
                                     Met Asn Ser Asp
                                     1
CCC GTC GTT TTA      126
Pro Val Val Leu
5

```

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

```

Met Asn Ser Asp Pro Val Val Leu
1              5

```

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-187-

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..63

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| GAA | ATC | CAG | CTG | AGC | GCC | GGT | CGC | TAC | CAT | TAC | CAG | TTG | GTC | TGG | TGT | 48 |
| Glu | Ile | Gln | Leu | Ser | Ala | Gly | Arg | Tyr | His | Tyr | Gln | Leu | Val | Trp | Cys |    |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |    |

|     |     |     |     |            |            |            |        |    |
|-----|-----|-----|-----|------------|------------|------------|--------|----|
| CAA | AAA | GAT | CCA | TAATTAATTA | ACCCGGGTCG | ACCTGCAGCC | TACATG | 96 |
| Gln | Lys | Asp | Pro |            |            |            |        |    |
|     |     |     | 20  |            |            |            |        |    |

## (2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ile | Gln | Leu | Ser | Ala | Gly | Arg | Tyr | His | Tyr | Gln | Leu | Val | Trp | Cys |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |

|     |     |     |     |
|-----|-----|-----|-----|
| Gln | Lys | Asp | Pro |
|     |     |     | 20  |

## (2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)



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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2

(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox virus

(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2

(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 124 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 104..124

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

```

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA      60
ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAA ATG AAT TCG CTA CTT      118
                               Met Asn Ser Leu Leu
                               1           5

GGA ACT                               124
Gly Thr

```

## (2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

```

Met Asn Ser Leu Leu Gly Thr
 1           5

```

## (2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 126 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

-190-

- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G
- (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1..12
- (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 103..126
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:
- |                                                                 |     |
|-----------------------------------------------------------------|-----|
| ATA AAA ATG TGATTAAAGTC TGAATGTGGA TCCATAATTA ATTAATTTTT        | 49  |
| Ile Lys Met                                                     |     |
| 1                                                               |     |
| ATCCCGGCGC GCCTCGACTC TAGAATTTC A TTTTGTTTTT TTCTATGCTA TAA ATG | 105 |
| Met                                                             |     |
| 1                                                               |     |
| AAT TCG GAT CCC GTC GTT TTA                                     | 126 |
| Asn Ser Asp Pro Val Val Leu                                     |     |
| 5                                                               |     |
- (2) INFORMATION FOR SEQ ID NO:89:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 3 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:
- |             |  |
|-------------|--|
| Ile Lys Met |  |
| 1           |  |
- (2) INFORMATION FOR SEQ ID NO:90:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Met Asn Ser Asp Pro Val Val Leu  
 1 5

## (2) INFORMATION FOR SEQ ID NO:91:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..63

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT | 48  |
| Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys |     |
| 1 5 10 15                                                       |     |
| CAA AAA GAT CCA TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG     | 100 |
| Gln Lys Asp Pro                                                 |     |
| 20                                                              |     |
| CAGGCGGCGC CTATAC                                               | 116 |

## (2) INFORMATION FOR SEQ ID NO:92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
 1 5 10 15

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Gln Lys Asp Pro  
20

## (2) INFORMATION FOR SEQ ID NO:93:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

## (2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

## (2) INFORMATION FOR SEQ ID NO:95:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..124

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGSCATATAA

60

ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAA ATG AAT TCC CCT GCC  
 Met Asn Ser Pro Ala  
 1 5

113

GCC CGG  
 Ala Arg

124

## (2) INFORMATION FOR SEQ ID NO:96:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Met Asn Ser Pro Ala Ala Arg

-194-

1

5

## (2) INFORMATION FOR SEQ ID NO:97:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 126 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..36
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 103..126

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

|                                                                  |     |
|------------------------------------------------------------------|-----|
| CTC CAG GAG CCC GCT CGC CTC GAG CGG GAT CCA TAATTAATTA ATTTTATCC | 53  |
| Leu Gln Glu Pro Ala Arg Leu Glu Arg Asp Pro                      |     |
| 1 5 10                                                           |     |
| CGGCGCGCCT CGACTCTAGA ATTCATTTT GTTTTTTCT ATGCTATAA ATG AAT      | 108 |
| Met Asn                                                          |     |
| 1                                                                |     |
| TCG GAT CCC GTC GTT TTA                                          | 126 |
| Ser Asp Pro Val Val Leu                                          |     |
| 5                                                                |     |

## (2) INFORMATION FOR SEQ ID NO:98:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 11 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

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Leu Gln Glu Pro Ala Arg Leu Glu Arg Asp Pro  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:99:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 8 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Met Asn Ser Asp Pro Val Val Leu  
 1 5

## (2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 116 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Swinepox virus  
 (B) STRAIN: Kasza  
 (C) INDIVIDUAL ISOLATE: S-SPV-001

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: 515-85.1

- (viii) POSITION IN GENOME:  
 (B) MAP POSITION: -23.2  
 (C) UNITS: %G

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1..63

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

|                                                                 |     |
|-----------------------------------------------------------------|-----|
| GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT | 48  |
| Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys |     |
| 1 5 10 15                                                       |     |
| CAA AAA GAT CCA TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG     | 100 |
| Gln Lys Asp Pro                                                 |     |
| 20                                                              |     |
| CAGGCGGCCG CTATAC                                               | 116 |



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(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys  
1 5 10 15

Gln Lys Asp Pro  
20

(2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Swinepox virus  
(B) STRAIN: Kasza  
(C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
(B) MAP POSITION: -23.2  
(C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

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- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

CCGAATTCCG GCTTCAGTAA CATAGGATCG

30

(2) INFORMATION FOR SEQ ID NO:104:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GTACCCATAC TGGTCGTGGC

20

(2) INFORMATION FOR SEQ ID NO:105:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

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- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

CCGGAATTGC CTACTTGGAA CTCTGG

26

(2) INFORMATION FOR SEQ ID NO:106:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

CATTGTCCCG AGACGGACAG

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(2) INFORMATION FOR SEQ ID NO:107:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

CGCGATCCAA CTATCGGTG

19

(2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GCGGATCCAC ATTCAGACTT AATCAC

26

(2) INFORMATION FOR SEQ ID NO:109:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double

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- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

ATGAATTCCC CTGCCGCCCG GACCGGCACC

30

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

CATGGATCCC GCTCGAGGCG AGCGGGCTCC

30

(2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

CTGGTTCGGC CCAGAATTCT ATGGGTCTCG CGCGGCTCGT GG

42

(2) INFORMATION FOR SEQ ID NO:112:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Swinepox virus
  - (B) STRAIN: Kasza
  - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: -23.2
  - (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

CTCGCTCGCC CAGGATCCCT AGCGGAGGAT GGAATTGAGT CG

42

(2) INFORMATION FOR SEQ ID NO:113:

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(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 3628 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Swinepox virus  
 (B) STRAIN: Kasza  
 (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
 (B) MAP POSITION: -23.2  
 (C) UNITS: %G

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 57..1226

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1362..3395

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| TTGAAGATGA ATGCATAGAG GAAGATGATG TCGANACGTC ATTATTTAAT GTATAAATGG  | 60  |
| ATAAATTGTA TCGCGCAATA TTCGGCGTTT TTATGACATC TAAAGATGAT GATTTTAATA  | 120 |
| ACTTTATAGA AGTTGTAAAA TCTGTATTAA CAGATACATC ANCTAATCAT ACAATATCGT  | 180 |
| CGTCCAATAA TAATACATGG ATATATATAT TTCTAGCGAT ATTATTTGGT GTTATGGNAT  | 240 |
| TATTAGTTTT TANTTTGTAT GTAGAAGTTC CTAAACCNAC TTANATGGAG GAAGCAGATA  | 300 |
| ACCNACTCGT TNTAAATAGT ATTAGTGCTA GAGCATTTGGN GGCATTTTTT GTATCTAAAA | 360 |
| NTANTGATAT GGTGNTGAA NTAGTTNCCC AAAAATNTCC NCCAAAGAAG ANATCACAAA   | 420 |
| TAAAACGCAT AGATACACGA ATTCCTATTG ATCTTATTAA TCAACAATTC GTTAAAAGAT  | 480 |
| TTAAACTAGA AAATTATAAA AATGGAATTT TATCCGTTCT TATCAATAGT TTAGTCGAAA  | 540 |
| ATAATTACTT TGAACAAGAT GGTAAACTTA ATAGCAGTGA TATTGATGAA TTAGTGCTCA  | 600 |
| CAGACATAGA GAAAAAGATT TTATCGTTGA TTCCTAGATG TTCTCCTCTT TATATAGATA  | 660 |
| TCAGTGACGT TAAAGTTCTC GCATCTAGGT TAANNAAAAG TGCTAAATCA TTTACGTTTA  | 720 |
| ATGATCATGA ATATATTATA CAATCTGATA AAATAGAGGA ATTAATAAAT AGTTTATCTA  | 780 |
| GAAACCATGA TATTATACTA GATGAAAAAA GTTCTATTAA AGACAGCATA TATATACTAT  | 840 |

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|             |             |             |             |            |             |      |
|-------------|-------------|-------------|-------------|------------|-------------|------|
| CTGATGATCT  | TTTGAATATA  | CTTCGTGAAA  | GATTATTTAG  | ATGTCCACAG | GTAAAGATA   | 900  |
| ATACTATTTT  | TAGAACACGT  | CTATATGATT  | ATTTTACTAG  | AGTGTCAAAG | AAAGAAGAAG  | 960  |
| CGAAAATATA  | CGTTATATTG  | AAAGATTTAA  | AGATTGCTGA  | TATACTCGGT | ATCGAAACAG  | 1020 |
| TAACGATAGG  | ATCATTTGTA  | TATACGAAAT  | ATAGCATGTT  | GATTAATTCA | ATTTCGTCTA  | 1080 |
| ATGTTGATAG  | ATATTCAAAA  | AGGTTCCATG  | ACTCTTTTTA  | TGAAGATATT | GCGGAATTTA  | 1140 |
| TAAAGGATAA  | TGAAAAAATT  | AATGTATCCA  | GAGTTGTTGA  | ATGCCTTATC | GTACCTAATA  | 1200 |
| TTAATATAGA  | GTTATTAACT  | GAATAAGTAT  | ATATAAATGA  | TTGTTTTTAT | AATGTTTGTT  | 1260 |
| ATCGCATTTA  | GTTTTGCTGT  | ATGGTTATCA  | TATACATTTT  | TAAGGCCGTA | TATGATAAAT  | 1320 |
| GAAAATATAT  | AAGCACTTAT  | TTTTGTAGT   | ATAATAACAC  | AATGCCGTCG | TATATGTATC  | 1380 |
| CGAAGAACGC  | AAGAAAAGTA  | ATTTCAAAGA  | TTATATCATT  | ACAACTTGAT | ATTAAAAAAC  | 1440 |
| TTCCTAAAAA  | ATATATAAAT  | ACCATGTTAG  | AATTTGGTCT  | ACATGGAAAT | CTACCAGCTT  | 1500 |
| GTATGTATAA  | AGATGCCGTA  | TCATATGATA  | TAAATAATAT  | AAGATTTTTA | CCTTATAATT  | 1560 |
| GTGTTATGGT  | TAAAGATTTA  | ATAAATGTTA  | TAAAATCATC  | ATCTGTAATA | GATACTAGAT  | 1620 |
| TACATCAATC  | TGTATTAAAA  | CATCGTAGAG  | CGTTAATAGA  | TTACGGCGAT | CAAGACATTA  | 1680 |
| TCACTTTAAT  | GATCATTAAAT | AAGTTACTAT  | CGATAGATGA  | TATATCCTAT | ATATTAGATA  | 1740 |
| AAAAAATAAT  | TCATGTAAAC  | AAAATATTAA  | AAATAGACCC  | TACAGTAGCC | AATTCAAACA  | 1800 |
| TGAAACTGAA  | TAAGATAGAG  | CTTGTAGATG  | TAATAACATC  | AATACCTAAG | TCTTCCTATA  | 1860 |
| CATATTTATA  | TAATAATATG  | ATCATTGATC  | TCGATACATT  | ATTATATTTA | TCCGATGCAT  | 1920 |
| TCCACATACC  | CCCCACACAT  | ATATCATTAC  | GTTCACTTAG  | AGATATAAAC | AGGATTATTG  | 1980 |
| AATTGCTTAA  | AAAATATCCG  | AATAATAATA  | TTATTGATTA  | TATATCCGAT | AGCATAAAAAT | 2040 |
| CAAAATAGTTC | ATTCATTAC   | ATACTTCATA  | TGATAATATC  | AAATATGTTT | CCTGCTATAA  | 2100 |
| TCCCTAGTGT  | AAACGATTTT  | ATATCTACCG  | TAGTTGATAA  | AGATCGACTT | ATTAATATGT  | 2160 |
| ATGGGATTAA  | GTGTGTTGCT  | ATGTTTTTCGT | ACGATATAAA  | CATGATCGAT | TTAGAGTCAT  | 2220 |
| TAGATGACTC  | AGATTACATA  | TTATAGAAA   | AAAATATATC  | TATATACGAC | GTTAAATGTA  | 2280 |
| GAGATTTTGC  | GAATATGATT  | AGAGATAAGG  | TTAAAAGAGA  | AAAGAATAGA | ATATTAACTA  | 2340 |
| CGAAATGTGA  | AGATATTATA  | AGATATATAA  | AATTATTCAG  | TAAAAATAGA | ATAAACGATG  | 2400 |
| AAAAATAATA  | GGTGGAGGAG  | GTGTTGATAC  | ATATTGATAA  | TGTATCTAAA | AATAATAAAT  | 2460 |
| TATCACTGTC  | TGATATATCA  | TCTTTAATGG  | ATCAATTTTCG | TTTAAATCCA | TGTACCATAA  | 2520 |
| GAAATATATT  | ATTATCTTCA  | GCAACTATAA  | AATCAAAACT  | ATTAGCGTTA | CGGGCAGTAA  | 2580 |
| AAAACCTGGA  | ATGTTATTCA  | TTGACAAATG  | TATCAATGTA  | TAAAAAATA  | AAGGGTGTTA  | 2640 |
| TCGTAATGGA  | TATGGTTGAT  | TATATATCTA  | CTAACATTCT  | TAAATACCAT | AAACAATTAT  | 2700 |



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|                                                                   |      |
|-------------------------------------------------------------------|------|
| ATGATAAAAT GAGTACGTTT GAATATAAAC GAGATATTAA ATCATGTAAA TGCTCGATAT | 2760 |
| GTTCGGACTC TATAACACAT CATATATATG AAACAACATC ATGTATAAAT TATAAATCTA | 2820 |
| CCGATAATGA TETTATGATA GTATTGTTCA ATCTAACTAG ATATTTAATG CATGGGATGA | 2880 |
| TACATCCTAA TCTTATAAGC GTAAAAGGAT GGGGTCCCCT TATTGGATTA TTAACGGGTG | 2940 |
| ATATAGGTAT TAATTTAAAA CTATATTCCA CCATGAATAT AAATGGGCTA CGGTATGGAG | 3000 |
| ATATTACGTT ATCTTCATAC GATATGAGTA ATAAATTAGT CTCTATTATT AATACACCCA | 3060 |
| TATATGAGTT AATACCGTTT ACTACATGTT GTTCACTCAA TGAATATTAT TCAAAAATTG | 3120 |
| TGATTTTAAT AAATGTTATT TTAGAATATA TGATATCTAT TATATTATAT AGAATATTGA | 3180 |
| TCGTAAAAAG ATTTAATAAC ATTAAAGAAT TTATTTCAAA AGTCGTAAAT ACTGTACTAG | 3240 |
| AATCATCAGG CATATATTTT TGTCAGATGC GTGTACATGA ACAAATTGAA TTGGAAATAG | 3300 |
| ATGAGCTCAT TATTAATGGA TCTATGCCTG TACAGCTTAT GCATTTACTT CTAAAGGTAG | 3360 |
| CTACCATAAT ATTAGAGGAA ATCAAAGAAA TATAACGTAT TTTTCTTTT AAATAAATAA  | 3420 |
| AAATACTTTT TTTTTTAAAC AAGGGGTGCT ACCTTGTCTA ATTGTATCTT GTATTTTGGA | 3480 |
| TCTGATGCAA GATTATTAAA TAATCGTATG AAAAAGTAGT AGATATAGTT TATATCGTTA | 3540 |
| CTGGACATGA TATTATGTTT AGTTAATTCT TCTTTGGCAT GAATTCTACA CGTCGGANAA | 3600 |
| GGTAATGTAT CTATAATGGT ATAAAGCT                                    | 3628 |

## (2) INFORMATION FOR SEQ ID NO:114:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

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Met Asp Lys Leu Tyr Ala Ala Ile Phe Gly Val Phe Met Thr Ser Lys  
 1 5 15  
 Asp Asp Asp Phe Asn Asn Phe Ile Glu Val Val Lys Ser Val Leu Thr  
 20 25 30  
 Asp Thr Ser Xaa Asn His Thr Ile Ser Ser Ser Asn Asn Asn Thr Trp  
 35 40 45  
 Ile Tyr Ile Phe Leu Ala Ile Leu Phe Gly Val Met Xaa Leu Leu Val  
 50 55 60  
 Phe Xaa Leu Tyr Val Glu Val Pro Lys Pro Thr Xaa Met Glu Glu Ala  
 65 70 75 80  
 Asp Asn Xaa Leu Val Xaa Asn Ser Ile Ser Ala Arg Ala Leu Xaa Ala  
 85 90 95  
 Phe Phe Val Ser Lys Xaa Xaa Asp Met Val Xaa Glu Xaa Val Xaa Gln  
 100 105 110  
 Lys Xaa Pro Pro Lys Lys Xaa Ser Gln Ile Lys Arg Ile Asp Thr Arg  
 115 120 125  
 Ile Pro Ile Asp Leu Ile Asn Gln Gln Phe Val Lys Arg Phe Lys Leu  
 130 135 140  
 Glu Asn Tyr Lys Asn Gly Ile Leu Ser Val Leu Ile Asn Ser Leu Val  
 145 150 155 160  
 Glu Asn Asn Tyr Phe Glu Gln Asp Gly Lys Leu Asn Ser Ser Asp Ile  
 165 170 175  
 Asp Glu Leu Val Leu Thr Asp Ile Glu Lys Lys Ile Leu Ser Leu Ile  
 180 185 190  
 Pro Arg Cys Ser Pro Leu Tyr Ile Asp Ile Ser Asp Val Lys Val Leu  
 195 200 205  
 Ala Ser Arg Leu Xaa Lys Ser Ala Lys Ser Phe Thr Phe Asn Asp His  
 210 215 220  
 Glu Tyr Ile Ile Gln Ser Asp Lys Ile Glu Glu Leu Ile Asn Ser Leu  
 225 230 235 240  
 Ser Arg Asn His Asp Ile Ile Leu Asp Glu Lys Ser Ser Ile Lys Asp  
 245 250 255  
 Ser Ile Tyr Ile Leu Ser Asp Asp Leu Leu Asn Ile Leu Arg Glu Arg  
 260 265 270  
 Leu Phe Arg Cys Pro Gln Val Lys Asp Asn Thr Ile Ser Arg Thr Arg  
 275 280 285  
 Leu Tyr Asp Tyr Phe Thr Arg Val Ser Lys Lys Glu Glu Ala Lys Ile  
 290 295 300  
 Tyr Val Ile Leu Lys Asp Leu Lys Ile Ala Asp Ile Leu Gly Ile Glu  
 305 310 315 320  
 Thr Val Thr Ile Gly Ser Phe Val Tyr Thr Lys Tyr Ser Met Leu Ile  
 325 330 335

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Asn Ser Ile Ser Ser Asn Val Asp Arg Tyr Ser Lys Arg Phe His Asp  
                   340                  345                  350

Ser Phe Tyr Glu Asp Ile Ala Glu Phe Ile Lys Asp Asn Glu Lys Ile  
                   355                  360                  365

Asn Val Ser Arg Val Val Glu Cys Leu Ile Val Pro Asn Ile Asn Ile  
                   370                  375                  380

Glu Leu Leu Thr Glu  
                   385

## (2) INFORMATION FOR SEQ ID NO:115:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 677 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Swinepox virus  
 (B) STRAIN: Kasza  
 (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:  
 (B) MAP POSITION: ~23.2  
 (C) UNITS: %G

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys  
 1                  5                  10                  15

Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile  
                   20                  25                  30

Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met  
                   35                  40                  45

Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro  
                   50                  55                  60

Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser  
                   65                  70                  75                  80

Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg  
                   85                  90                  95

Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile  
                   100                  105                  110

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Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys  
 115 120 125  
 Ile Ile His Val Thr Lys Ile Leu Lys Ile Asp Pro Thr Val Ala Asn  
 130 135 140  
 Ser Asn Met Lys Leu Asn Lys Ile Glu Leu Val Asp Val Ile Thr Ser  
 145 150 155 160  
 Ile Pro Lys Ser Ser Tyr Thr Tyr Leu Tyr Asn Asn Met Ile Ile Asp  
 165 170 175  
 Leu Asp Thr Leu Leu Tyr Leu Ser Asp Ala Phe His Ile Pro Pro Thr  
 180 185 190  
 His Ile Ser Leu Arg Ser Leu Arg Asp Ile Asn Arg Ile Ile Glu Leu  
 195 200 205  
 Leu Lys Lys Tyr Pro Asn Asn Asn Ile Ile Asp Tyr Ile Ser Asp Ser  
 210 215 220  
 Ile Lys Ser Asn Ser Ser Phe Ile His Ile Leu His Met Ile Ile Ser  
 225 230 235 240  
 Asn Met Phe Pro Ala Ile Ile Pro Ser Val Asn Asp Phe Ile Ser Thr  
 245 250 255  
 Val Val Asp Lys Asp Arg Leu Ile Asn Met Tyr Gly Ile Lys Cys Val  
 260 265 270  
 Ala Met Phe Ser Tyr Asp Ile Asn Met Ile Asp Leu Glu Ser Leu Asp  
 275 280 285  
 Asp Ser Asp Tyr Ile Phe Ile Glu Lys Asn Ile Ser Ile Tyr Asp Val  
 290 295 300  
 Lys Cys Arg Asp Phe Ala Asn Met Ile Arg Asp Lys Val Lys Arg Glu  
 305 310 315 320  
 Lys Asn Arg Ile Leu Thr Thr Lys Cys Glu Asp Ile Ile Arg Tyr Ile  
 325 330 335  
 Lys Leu Phe Ser Lys Asn Arg Ile Asn Asp Glu Asn Asn Lys Val Glu  
 340 345 350  
 Glu Val Leu Ile His Ile Asp Asn Val Ser Lys Asn Asn Lys Leu Ser  
 355 360 365  
 Leu Ser Asp Ile Ser Ser Leu Met Asp Gln Phe Arg Leu Asn Pro Cys  
 370 375 380  
 Thr Ile Arg Asn Ile Leu Leu Ser Ser Ala Thr Ile Lys Ser Lys Leu  
 385 390 395 400  
 Leu Ala Leu Arg Ala Val Lys Asn Trp Lys Cys Tyr Ser Leu Thr Asn  
 405 410 415  
 Val Ser Met Tyr Lys Lys Ile Lys Gly Val Ile Val Met Asp Met Val  
 420 425 430  
 Asp Tyr Ile Ser Thr Asn Ile Leu Lys Tyr His Lys Gln Leu Tyr Asp  
 435 440 445

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Lys Met Ser Thr Phe Glu Tyr Lys Arg Asp Ile Lys Ser Cys Lys Cys  
 450 455 460  
 Ser Ile Cys Ser Asp Ser Ile Thr His His Ile Tyr Glu Thr Thr Ser  
 465 470 475 480  
 Cys Ile Asn Tyr Lys Ser Thr Asp Asn Asp Leu Met Ile Val Leu Phe  
 485 490 495  
 Asn Leu Thr Arg Tyr Leu Met His Gly Met Ile His Pro Asn Leu Ile  
 500 505 510  
 Ser Val Lys Gly Trp Gly Pro Leu Ile Gly Leu Leu Thr Gly Asp Ile  
 515 520 525  
 Gly Ile Asn Leu Lys Leu Tyr Ser Thr Met Asn Ile Asn Gly Leu Arg  
 530 535 540  
 Tyr Gly Asp Ile Thr Leu Ser Ser Tyr Asp Met Ser Asn Lys Leu Val  
 545 550 555 560  
 Ser Ile Ile Asn Thr Pro Ile Tyr Glu Leu Ile Pro Phe Thr Thr Cys  
 565 570 575  
 Cys Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val  
 580 585 590  
 Ile Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val  
 595 600 605  
 Lys Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr  
 610 615 620  
 Val Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu  
 625 630 635 640  
 Gln Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro  
 645 650 655  
 Val Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu  
 660 665 670  
 Glu Ile Lys Glu Ile  
 675

## (2) INFORMATION FOR SEQ ID NO:116:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Infectious bovine rhinotracheitis virus
- (B) STRAIN: Cooper Strain

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

CTGGTTCGGC CCAGAAATTCG ATGCAACCCA CCGCGCCGCC CCG

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(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Infectious bovine rhinotracheitis virus
- (B) STRAIN: Cooper Strain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

CTCGCTCGCC CAGGATCCCT AGCGGAGGAT GGACTTGAGT CG

42

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Equine Influenza A neuraminidase
- (B) STRAIN: Prague/56

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GGGATCCATG AATCCTAATC AAAAATCTT T

31

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

-210-

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Equine Influenza A neuraminidase
  - (B) STRAIN: Prague/56

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

GGGATCCTTA CGAAAAGTAT TTAATTTGTG C

31

(2) INFORMATION FOR SEQ ID NO:120:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 42 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Equine influenza A hemagglutinin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

GGAGGCCTTC ATGACAGACA ACCATTATTT TGATACTACT GA

42

(2) INFORMATION FOR SEQ ID NO:121:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Equine influenza A hemagglutinin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

GAAGGCCTTC TCAAATGCAA ATGTTGCATC TGATGTTGCC

40

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## (2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 32 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Equine Influenza A hemagglutinin
  - (B) STRAIN: Prague/56

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GGGATCCATG AACACTCAAA TTCTAATATT AG

32

## (2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Equine Influenza A hemagglutinin
  - (B) STRAIN: Prague/56

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

GGGATCCTTA TATACAAATA GTGCACCGCA

30

## (2) INFORMATION FOR SEQ ID NO:124:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO



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- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Equine Influenza A neuraminidase

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

GGGTCGACAT GAATCCAAAT CAAAAGATAA

30

- (2) INFORMATION FOR SEQ ID NO:125:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Equine Influenza A neuraminidase

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

GGGTCGACTT ACATCTTATC GATGTCAAA

29

- (2) INFORMATION FOR SEQ ID NO:126:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Human

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

CTCGAATTCG AAGTGGGCAA CGTGGATCCT CGC

33

- (2) INFORMATION FOR SEQ ID NO:127:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

CAGTTAGCCT CCCCCATCTC CCCA

24

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Equine herpesvirus type 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

CGGAATTCCT CTGGTTGCCG T

21

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Equine herpesvirus type 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

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GACGGTGGAT CCGGTAGGCG GT

22

## (2) INFORMATION FOR SEQ ID NO:130:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bovine parainfluenza-3 virus

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

TTATGGATCC TGCTGCTGTG TTGAACAACT TTGT

34

## (2) INFORMATION FOR SEQ ID NO:131:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bovine parainfluenza-3 virus

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

CCGCGGATCC CATGACCATC ACAACCATAA TCATAGCC

38

## (2) INFORMATION FOR SEQ ID NO:132:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

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- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Bovine parainfluenza-3 virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:  
CGTCGGATCC CTTAGCTGCA GTTTTTTGGG ACTTCTGTTT TGA

43

(2) INFORMATION FOR SEQ ID NO:133:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Bovine parainfluenza-3 virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:  
CATAGGATCC CATGGAATAT TGGAAACACA CAAACAGCAC

40

(2) INFORMATION FOR SEQ ID NO:134:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Bovine viral diarrhea virus  
(B) STRAIN: Singer Strain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:  
ACGTCGGATC CCTTACCAAA CCACGTCTTA CTCTGTGTTT CC

42

(2) INFORMATION FOR SEQ ID NO:135:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double

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- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Bovine viral diarrhea virus
  - (B) STRAIN: Singer Strain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

ACATAGGATC CCATGGGAGA AACATAACA CAGTGAACC

40

(2) INFORMATION FOR SEQ ID NO:136:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 33 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Bovine viral diarrhea virus
  - (B) STRAIN: Singer Strain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

CGTGGATCCT CAATTACAAG AGGTATCGTC TAC

33

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Bovine viral diarrhea virus
  - (B) STRAIN: Singer Strain

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

CATAGATCTT GTGGTGCTGT CCGACTTCGC A

31

## (2) INFORMATION FOR SEQ ID NO:138:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bovine respiratory syncytial virus
- (B) STRAIN: Strain 375

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

TGCAGGATCC TCATTACTA AAGGAAAGAT TGTTGAT

37

## (2) INFORMATION FOR SEQ ID NO:139:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bovine respiratory syncytial virus
- (B) STRAIN: Strain 375

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

CTCTGGATCC TACAGCCATG AGGATGATCA TCAGC

35

## (2) INFORMATION FOR SEQ ID NO:140:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bovine respiratory syncytial virus

(B) STRAIN: Strain 375

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

CGTCGGATCC CTCACAGTTC CACATCATTTG TCTTTGGGAT

40

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bovine respiratory syncytial virus

(B) STRAIN: Strain 375

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

CTTAGGATCC CATGGCTCTT AGCAAGGTCA AACTAAATGA C

41

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bovine respiratory syncytial virus

(B) STRAIN: Strain 375

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

CGTTGGATCC CTAGATCTGT GTAGTTGATT GATTTGTGTG A

41

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## (2) INFORMATION FOR SEQ ID NO:143:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 41 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Bovine respiratory syncytial virus
  - (B) STRAIN: Strain 375

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

CTCTGGATCC TCATACCCAT CATCTTAAAT TCAAGACATT A

41

## (2) INFORMATION FOR SEQ ID NO:144:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

## (2) INFORMATION FOR SEQ ID NO:145:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 128 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:



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|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA | 60  |
| ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT TGATCCATGA | 120 |
| ATCCTAAT                                                          | 128 |

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## (2) INFORMATION FOR SEQ ID NO:146:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 120 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

```
CTTTTCGTAA GGATCAATTC GGATCCATAA TTAATTAATT TTTATCCCGG CGCGCCTCGA    60
CTCTAGAATT TCATTTTGTT TTTTCTATG CTATAAATGA ATTCGGATCC CGTCGTTTTA    120
```

## (2) INFORMATION FOR SEQ ID NO:147:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 116 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

```
GAAATCCAGC TGAGCGCCGG TCGTACCAT TACCAGTTGG TCTGGTGTCA
AAAAGATCCA    60
```

```
TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC
116
```

## (2) INFORMATION FOR SEQ ID NO:148:

-222-

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C  
51

## (2) INFORMATION FOR SEQ ID NO:149:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

## (2) INFORMATION FOR SEQ ID NO:150:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 168 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

```
GTATTGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA    60
ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CACCCGCTGG    120
TGGCGGTCTT TGGCGCGGGC CCCGTGGGCA TCGGCCCGGG CACCACGG    168
```

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 112 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

```
GAGCTCGAAT TCGGATCCAT AATTAATTAA TTTTATCCC GGCGCGCCTC GACTCTAGAA    60
TTTCATTTTG TTTTCTTCTA TGCTATAAAT GAATTCGGAT CCCGTCGTTT TA    112
```

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA  
AAAAGATCCA 60

TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC  
116

## (2) INFORMATION FOR SEQ ID NO:153:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

## (2) INFORMATION FOR SEQ ID NO:154:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

## (2) INFORMATION FOR SEQ ID NO:155:

-225-

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 104 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| AAATATATAA ATACCATGTT AGAATTTGGT CTGCTGCAGG TCGACTCTAG AATTTTCATTT | 60  |
| TGTTTTTTTTC TATGCTATAA ATGAATTCGG ATCCCGTCGT TTTA                  | 104 |

## (2) INFORMATION FOR SEQ ID NO:156:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 185 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTC AAAAGATCCA  | 60  |
| TAATTAATTA ACCCGGTCGA CTCTAGAAAA AATTGAAAA CTATTCTAAT TTATTGCACG  | 120 |
| GAGATCTTTT TTTTTTTTTT TTTTTTGCCA TATAAATGAA TTCGGATCCC CGGTGGCTTT | 180 |
| GGGGG                                                             | 185 |

## (2) INFORMATION FOR SEQ ID NO:157:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 66 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

CTCAATGTTA GGGTACCGAG CTCGAATTGG GTCGACCGGG TCGACCTGCA GCCTACATGG  
60

AAATCT

66

(2) INFORMATION FOR SEQ ID NO:158:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

(2) INFORMATION FOR SEQ ID NO:159:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 51 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

(2) INFORMATION FOR SEQ ID NO:160:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 127 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA 60  
ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT TCGACATGAA 120  
TCCAAAT 127

(2) INFORMATION FOR SEQ ID NO:161:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 122 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

GATAAGATGT AAGTCGAAAT TCGGATCCAT AATTAATTAA TTTTATCCC GCGCGCCTC 60  
GACTCTAGAA TTTCATTTTG TTTTTTCTA TGCTATAAAT GAATTCGGAT CCCGTCGTTT 120



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TA

122

## (2) INFORMATION FOR SEQ ID NO:162:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

```
GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCA GTTGG TCTGGTGTCA AAAAGATCCA      60
TAATTAATTA ACCCGGGTCG AGGCGGCCCG GGTGACCTG CAGGCGGCCG CTATAC      116
```

## (2) INFORMATION FOR SEQ ID NO:163:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

```
TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C      51
```

## (2) INFORMATION FOR SEQ ID NO:164:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 61 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

GTATAGCGGC CGCCTGCAGG TCGACCTGCA GTGAATAATA AAATGTGTGT TTGTCCGAAA 60  
T 61

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 45 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

CTCCATAGAA GACACCGGGA CCATGGATCC CGTCGTTTTA CAACG 45

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:

- 230 -

- (A) LENGTH: 105 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| TCGGCGGAAA TCCAGCTGAG CGCCGGTCGC TACCATTACC AGTTGGTCTG GTGTCAAAAA | 60  |
| GATCTAGAAT AAGCTAGAGG ATCGATCCCC TATGGCGATC ATCAG                 | 105 |

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

|                                    |    |
|------------------------------------|----|
| CTGCAGGTGC ACCTGCAGGC GGCCGCTATA C | 31 |
|------------------------------------|----|

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid

- 231 -

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C

51

(2) INFORMATION FOR SEQ ID NO:170:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T

51

(2) INFORMATION FOR SEQ ID NO:171:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 193 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA | 60  |
| ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CCGAAGTGGG | 120 |
| CAACGTGGAT CCTCGCCCTC GGGCTCCTCG TGGTCCGCAC CGTCGTGGCC AGAAGTGCTC | 180 |
| CTACTAGCTC GAG                                                    | 193 |

(2) INFORMATION FOR SEQ ID NO:172:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

|                                                                  |     |
|------------------------------------------------------------------|-----|
| ATCATTAGCA CGTTAACTTA ATAAGATCCA TAATTAATTA ATTTTATCC CGGCGCGCCT | 60  |
| CGACTCTAGA ATTCATTTT GTTTTTTCT ATGCTATAAA TGAATTCGGA TCCCGTCGTT  | 120 |
| TTA                                                              | 123 |

(2) INFORMATION FOR SEQ ID NO:173:

- 233 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA  
AAAAGATCCA 60

TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC  
116

## (2) INFORMATION FOR SEQ ID NO:174:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C  
51

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## (2) INFORMATION FOR SEQ ID NO:175:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

## (2) INFORMATION FOR SEQ ID NO:176:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 133 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTTT TTTTTTTTTT TGGCATATAA 60  
ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CCTCTGGTTG 120  
CCGTTCTGTC GGC 133

## (2) INFORMATION FOR SEQ ID NO:177:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

- 235 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:

```
GAAAATGAAA AAATGGTTTA AACCGGGGGC GCGCCTCGAC TCTAGAATTT CATTTTGTTT    60
TTTCTATGCT TATAAATGAA TTCGGATCCC GTCGTTTTTA    99
```

(2) INFORMATION FOR SEQ ID NO:178:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 116 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

```
GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA
AAAAGATCCA    60
```

```
TAATTAATTA ACCCGGGTCG AGGCGCGCCG GGTCGACCTG CAGGCGGCCG CTATAC
116
```

(2) INFORMATION FOR SEQ ID NO:179:

(i) SEQUENCE CHARACTERISTICS:



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- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C  
51

(2) INFORMATION FOR SEQ ID NO:180:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:

ACAGGAAACA GCTATGACCA TGATTACGAA TTCGAGCTCG CCCGGGGATC T 51

(2) INFORMATION FOR SEQ ID NO:181:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 140 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GTATAGCGGC CGCCTGCAGG TCGACTCTAG ATTTTTTTTT TTTTTTTTTT TGGCATATAA | 60  |
| ATAGATCTGT ATCCTAAAAT TGAATTGTAA TTATCGATAA TAAATGAATT CGGATCAGCT | 120 |
| TATGATGGAT GGACGTTTGG                                             | 140 |

## (2) INFORMATION FOR SEQ ID NO:182:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:

|                                                                  |     |
|------------------------------------------------------------------|-----|
| GGAGGTGTCC ACGGCCTTAA AGCTGATCCA TAATTAATTA ATTTTATCC CGGCGCGCCT | 60  |
| CGACTCTAGA ATTTCATTTT GTTTTTTCT ATGCTATAAA TGAATTCGGA TCCCGTCGTT | 120 |
| TTA                                                              | 123 |

## (2) INFORMATION FOR SEQ ID NO:183:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 116 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

GAAATCCAGC TGAGCGCCGG TCGCTACCAT TACCAGTTGG TCTGGTGTCA AAAAGATCCA 60  
TAATTAATTA ACCCGGGTCG AGGCGGCCCG GGTGACCTG CAGGCGGCCG CTATAC 116

## (2) INFORMATION FOR SEQ ID NO:184:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

TAATGTATCT ATAATGGTAT AAAGCTTGTA TTCTATAGTG TCACCTAAAT C 51

## (2) INFORMATION FOR SEQ ID NO:185:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

GAAGCATGCC CGTTCTTATC AATAGTTTAG TCGAAAATA 39

## (2) INFORMATION FOR SEQ ID NO:186:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

CATAAGATCT GGCATTGTGT TATTATACTA ACAAAAATAA G

41

(2) INFORMATION FOR SEQ ID NO:187:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

CCGTAGTCGA CAAAGATCGA CTTATTAATA TGTATGGGAT T

41

(2) INFORMATION FOR SEQ ID NO:188:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

GCCTGAAGCT TCTAGTACAG TATTTACGAC TTTTGAAAT

39

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What is claimed is:

1. A recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.
2. The recombinant swinepox virus of claim 1, wherein the antigenic polypeptide is derived from the group consisting of human herpesvirus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, human immunodeficiency virus, rabies virus, measles virus, hepatitis B virus and hepatitis C virus.
3. The recombinant swinepox virus of claim 1, wherein the antigenic polypeptide is associated with malaria or malignant tumor from the group consisting of *Plasmodium falciparum*, *Bordetella pertussis*, and malignant tumor.
4. The recombinant swinepox virus of claim 2, wherein the antigenic polypeptide is hepatitis B virus core protein or hepatitis B virus surface protein.
5. The recombinant swinepox virus of claim 4, designated S-SPV-031.
6. A recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential

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site of the swinepox genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune response in a host infected by the recombinant swinepox virus and is capable of being expressed in the host.

7. The recombinant swinepox virus of claim 6, wherein the cytokine is selected from a group consisting of interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin receptors.
8. The recombinant swinepox virus of claim 7, wherein the cytokine is human interleukin-2.
9. The recombinant swinepox virus of claim 8, designated S-SPV-035.
10. A recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant swinepox virus.
11. The recombinant swinepox virus of claim 10, wherein the antigenic polypeptide is derived from equine influenza virus or equine herpesvirus.
12. The recombinant swinepox virus of claim 11, wherein the antigenic polypeptide is selected from the group consisting of equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza

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virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.

13. The recombinant swinepox virus of claim 12, wherein the antigenic polypeptide is equine influenza virus type A/Alaska 91 neuraminidase.
14. The recombinant swinepox virus of claim 13, designated S-SPV-033.
15. The recombinant swinepox virus of claim 12, wherein the antigenic polypeptide is equine influenza virus type A/Prague 56 neuraminidase.
16. The recombinant swinepox virus of claim 15, designated S-SPV-034.
17. The recombinant swinepox virus of claim 12, wherein the antigenic polypeptide is equine herpesvirus type 1 glycoprotein B.
18. The recombinant swinepox virus of claim 17, designated S-SPV-038.
19. The recombinant swinepox virus of claim 12, wherein the antigenic polypeptide is equine herpesvirus type 1 glycoprotein D.
20. The recombinant swinepox virus of claim 19, designated S-SPV-039.
21. A recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential



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site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.

22. The recombinant swinepox virus of claim 21, wherein the antigenic polypeptide is selected from the group consisting of bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.
23. The recombinant swinepox virus of claim 22, wherein the antigenic polypeptide is the bovine respiratory syncytial virus attachment protein (BRSV G).
24. The recombinant swinepox virus of claim 23, designated S-SPV-020.
25. The recombinant swinepox virus of claim 22, wherein the antigenic polypeptide is the bovine respiratory syncytial virus fusion protein (BRSV F).
26. The recombinant swinepox virus of claim 25, designated S-SPV-029.
27. The recombinant swinepox virus of claim 22, wherein the antigenic polypeptide is the bovine respiratory syncytial virus nucleocapsid protein (BRSV N).

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28. The recombinant swinepox virus of claim 27, designated S-SPV-030.
29. The recombinant swinepox virus of claim 22, wherein the antigenic polypeptide is the bovine parainfluenza virus type 3 fusion protein.
30. The recombinant swinepox virus of claim 33, designated S-SPV-028.
31. The recombinant swinepox virus of claim 21, wherein the antigenic polypeptide is the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.
32. The recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome wherein the foreign DNA sequence encodes the bovine viral diarrhea virus glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.
33. The recombinant swinepox virus of claim 32, designated S-SPV-032.
34. The recombinant swinepox virus of claim 32, designated S-SPV-040.
35. A recombinant swinepox virus which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease virus and

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wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox virus.

36. The recombinant swinepox virus of claim 35, wherein the antigenic polypeptide is the infectious bursal disease virus polyprotein.
37. The recombinant swinepox virus of claim 36, designated S-SPV-026.
38. The recombinant swinepox virus of claim 35, wherein the antigenic polypeptide is the infectious bursal disease virus VP2.
39. The recombinant swinepox virus of claim 38, designated S-SPV-027.
40. The recombinant swinepox virus of claim 1, 6, 10, 21, 32 or 35, wherein the insertion site is present within the larger *HindIII* to *BglIII* sub-fragment of the *HindIII* M fragment of the swinepox viral genome.
41. The recombinant swinepox virus of claim 40, wherein the insertion site is within an open reading frame contained in the *HindIII* to *BglIII* sub-fragment.
42. The recombinant swinepox virus of claim 41, wherein the insertion site is the *AccI* restriction endonuclease site located in the *HindIII* to *BglIII* sub-fragment.

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43. The recombinant swinepox virus of claim 42, wherein the *AccI* restriction endonuclease site is replaced by a *NotI* restriction endonuclease site.
44. The recombinant swinepox virus of claim 42, wherein the *AccI* restriction endonuclease site is replaced by a *PstI* restriction endonuclease site.
45. The recombinant swinepox virus of claim 1, 6, 10, 21, 32 or 35, wherein the insertion site is present within an open reading frame encoding swinepox virus thymidine kinase.
46. The recombinant swinepox virus of claim 45, wherein the insertion site is the *NdeI* restriction endonuclease site located within the open reading frame encoding the swinepox virus thymidine kinase.
47. The recombinant swinepox virus of claim 46, wherein the *NdeI* restriction site is replaced by a *AscI* restriction endonuclease site.
48. The recombinant swinepox virus of claim 1, 6, 10, 21, 32 or 35, wherein the expression of the foreign DNA sequence is under control of a promoter located upstream of the foreign DNA sequence.
49. The recombinant swinepox virus of claim 48, wherein the promoter is an endogenous swinepox viral promoter or an exogenous promoter.
50. The recombinant swinepox virus of claim 49, wherein the exogenous promoter is a synthetic pox viral promoter.

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51. The recombinant swinepox virus of claim 49, wherein the exogenous promoter is human cytomegalovirus immediately early gene promoter.
52. A homology vector for producing a recombinant swinepox virus by inserting a foreign DNA sequence into the swinepox virus genome which comprises a double-stranded DNA molecule consisting of:
  - a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from a human pathogen;
  - b) at one end of the foreign DNA sequence, double-stranded swinepox virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the swinepox genomic DNA;
  - c) at the other end of the foreign DNA sequence, double stranded swinepox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.
53. The homology vector of claim 52, wherein the antigenic polypeptide is hepatitis B virus core protein.
54. A homology vector for producing a recombinant swinepox virus by inserting a foreign DNA sequence into the swinepox virus genome which comprises a double-stranded DNA molecule consisting of:
  - a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from a

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cytokine capable of stimulating human immune response;

- b) at one end of the foreign DNA sequence, double-stranded swinepox virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the swinepox genomic DNA;
  - c) at the other end of the foreign DNA sequence, double stranded swinepox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.
55. The homology vector of claim 54, wherein the cytokine is interleukin-2.
56. A homology vector for producing a recombinant swinepox virus by inserting a foreign DNA sequence into the swinepox virus genome which comprises a double-stranded DNA molecule consisting of:
- a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from an equine pathogen;
  - b) at one end of the foreign DNA sequence, double-stranded swinepox virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the swinepox genomic DNA;
  - c) at the other end of the foreign DNA sequence, double stranded swinepox virus genomic DNA

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homologous to the genomic DNA located at the other side of the same site.

57. The homology vector of claim 56, wherein the antigenic polypeptide is selected from the group consisting of equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Alaska 81 neuraminidase, equine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.
58. A homology vector for producing a recombinant swinepox virus by inserting a foreign DNA sequence into the swinepox virus genome which comprises a double-stranded DNA molecule consisting of:
  - a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus;
  - b) at one end of the foreign DNA sequence, double-stranded swinepox virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the swinepox genomic DNA;
  - c) at the other end of the foreign DNA sequence, double stranded swinepox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.

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59. The homology vector of claim 58, wherein the antigenic polypeptide is selected from the group consisting of bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.
60. A homology vector for producing a recombinant swinepox virus by inserting foreign DNA sequence into the swinepox virus genome which comprises a double-stranded DNA molecule consisting of:
- a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from infectious bursal disease virus;
  - b) at one end of the foreign DNA sequence, double-stranded swinepox virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the swinepox genomic DNA;
  - c) at the other end of the foreign DNA sequence, double stranded swinepox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.
61. The homology vector of claim 60, wherein the antigenic polypeptide is infectious bursal disease virus polyprotein or infectious bursal disease virus VP2.



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62. The homology vector of claim 52, 54, 56, 58, or 60, wherein the double-stranded swinepox virus genomic DNA is homologous to the genomic DNA present within the larger *HindIII* to *BglII* sub-fragment of the *HindIII* M fragment of the swinepox virus genome.
63. The homology vector of claim 62, wherein the double-stranded swinepox virus genomic DNA is homologous to the genomic DNA present within the open reading frame contained in the *HindIII* to *BglII* sub-fragment.
64. The homology vector of claim 62, wherein the non-essential site is the *AccI* restriction endonuclease site located within the *HindIII* to *BglII* sub-fragment.
65. The homology vector of claim 52, 54, 56, 58, or 60, wherein the double-stranded swinepox virus genomic DNA is homologous to an open reading frame encoding swinepox virus thymidine kinase.
66. The homology vector of claim 65, wherein the non-essential site is the *NdeI* restriction endonuclease site located within the open reading frame encoding swinepox virus thymidine kinase.
67. The homology vector of claim 52, 54, 56, 58, or 60, wherein the foreign DNA sequence is under control of a promoter located upstream of the foreign DNA sequence.
68. The homology vector of claim 67, wherein the promoter is an endogenous swinepox viral promoter or an exogenous promoter.

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69. The homology vector of claim 67, wherein the exogenous promoter is a synthetic pox viral promoter.
70. The homology vector of claim 69, wherein the exogenous promoter is human cytomegalovirus immediate early gene promoter.
71. A vaccine against a human pathogen which comprises an effective immunizing amount of the recombinant swinepox virus of claim 1 and a suitable carrier.
72. A vaccine against an equine pathogen which comprises an effective immunizing amount of the recombinant swinepox virus of claim 10 and a suitable carrier.
73. A vaccine against equine influenza virus type A which comprises an effective immunizing amount of the recombinant swinepox virus of claim 13 or 15 and a suitable carrier.
74. A vaccine against equine herpesvirus type 1 which comprises an effective immunizing amount of the recombinant swinepox virus of claim 17 or 19 and a suitable carrier.
75. A vaccine against bovine respiratory syncytial virus which comprises an effective immunizing amount of the recombinant swinepox virus of claim 21, 23, 25, or 27 and a suitable carrier.
76. A vaccine against bovine parainfluenza virus type 3 which comprises an effective immunizing amount of

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the recombinant swinepox virus of claim 29 or 31 and a suitable carrier.

77. A vaccine against bovine viral diarrhea virus which comprises an effective immunizing amount of the recombinant swinepox virus of claim 32 and a suitable carrier.
78. A vaccine against infectious bursal disease virus which comprises an effective immunizing amount of the recombinant swinepox virus of claim 36 or 38 and a suitable carrier.
79. A method of immunizing an animal against a human pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 71.
80. A method of immunizing an animal against an equine pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 72.
81. A method of immunizing an animal against equine influenza virus type A which comprises administering to the animal an effective immunizing dose of the vaccine of claim 73.
82. A method of immunizing an animal against equine herpesvirus type 1 which comprises administering to the animal an effective immunizing dose of the vaccine of claim 74.
83. A method of immunizing an animal against bovine respiratory syncytial virus which comprises

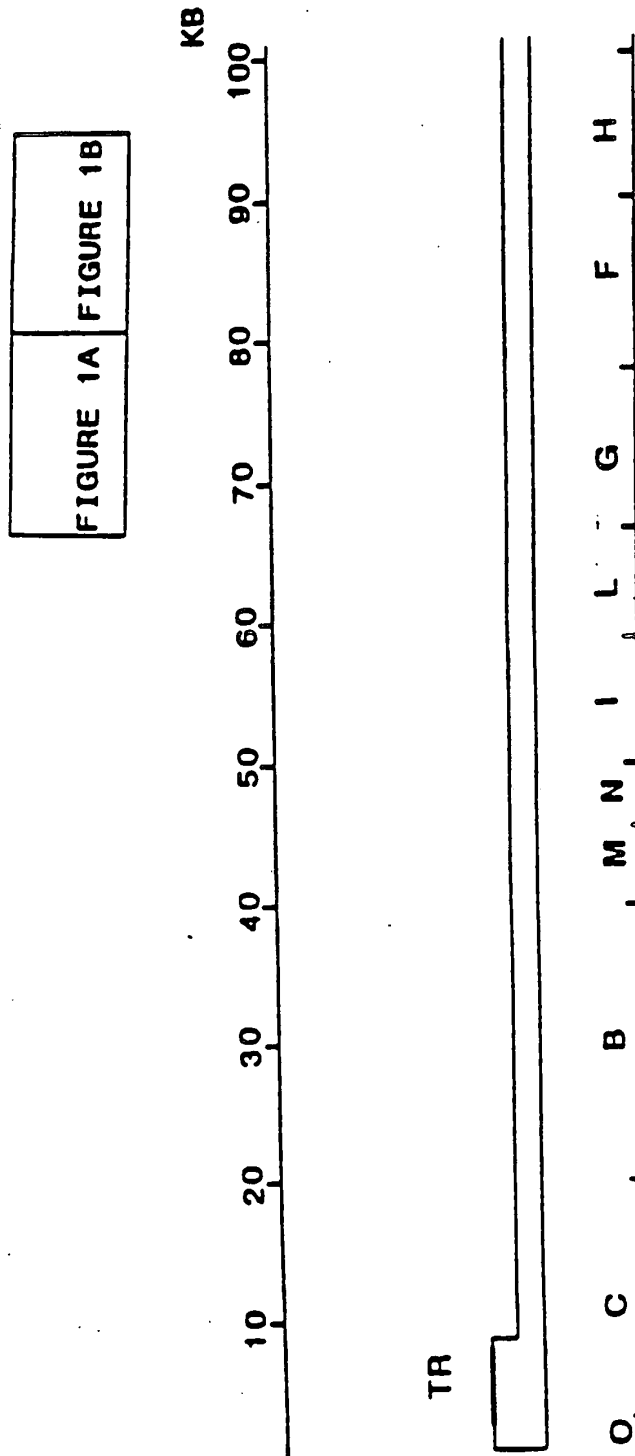
-255-

administering to the animal an effective immunizing dose of the vaccine of claim 75.

84. A method of immunizing an animal against bovine parainfluenza virus type 3 which comprises administering to the animal an effective immunizing dose of the vaccine of claim 76.
85. A method of immunizing an animal against bovine viral diarrhea virus which comprises administering to the animal an effective immunizing dose of the vaccine of claim 77.
86. A method of immunizing an animal against infectious bursal disease virus which comprises administering to the animal an effective immunizing dose of the vaccine of claim 78.
87. A method of enhancing human immune response which comprises administering to a person an effective dose of a recombinant swinepox virus of claim 6, 7 or 8 and a suitable carrier.

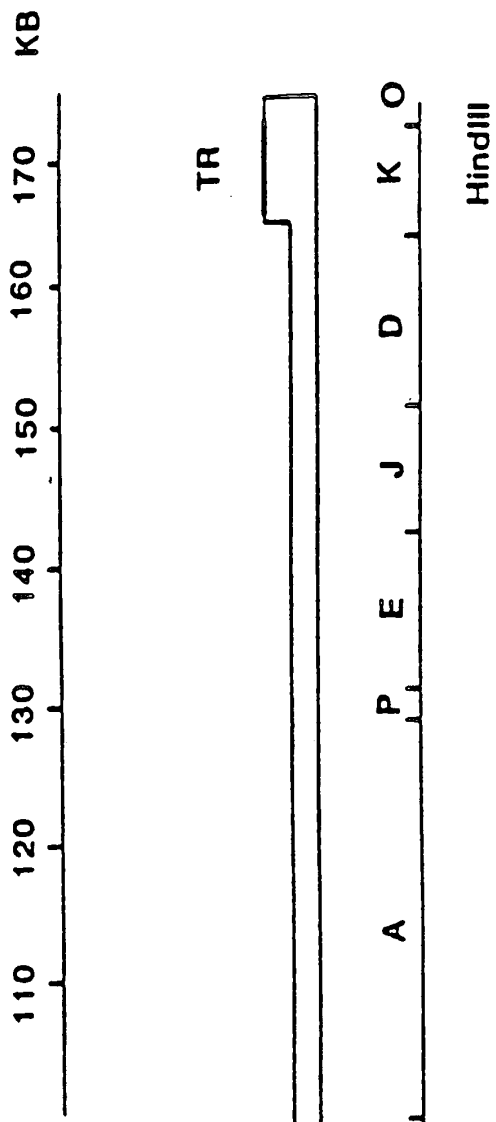
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FIGURE 1A



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FIGURE 1B



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FIGURE 2A

|           |
|-----------|
| FIGURE 2A |
| FIGURE 2B |

AATGTATCCAGAGTTGTTGAATGCCCTTATCGTACCTAATATTAATATAGAGTTATTAACT  
GAATAAGTATATATAAATGATTGTTTTTATAATGTTGTTATCGCATTTAGTTTTGCTGT  
ATGGTTATCATATACATTTTTTAAGGCCGTATATGATAAATGAAATATATAAGCACATTAT  
TTTTGTTAGTATAATACACAATGCGCGTATATGTATCCGAAGACGCAAGAAAGTA  
ATTCAAAGATTATATCATTTACAACTTGATATTAAAACTTCCTAAATAATATATAAAT  
ACCATGTTAGAAATTTGGTCTACATGGAAATCTACCAGCTTGTATGTATAAAGATGCCGTA  
MetProSer.....  
TCATATGATATAAATAATATAAGATTTTTTACCTTATAATTGTGTATGGTTAAAGATTTA  
ATAAATGTTATAAATCATCATCTGTAAATAGATAGATTACATCAATCTGTATTAAAA  
CATCGTAGAGCGTTAATAGATTACGGCGATCAAGACATTTATCACTTTAATGATCATTAAT  
AAGTTACTATCGATAGATGATATATCCCTATATATTAGATAAAAAAATAATTCATGTAAAC  
.....IleHisVal

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FIGURE 2B

GAGATATTAAATCATGTAAATGCTCGATATGTTCCGACTCTATAACACATCATATATATG  
AspIleLysSer.....  
AAACAACATCATGTATAAATTATAAATCTACCGATAAATGATCTTATGATAGTATTGTTCA  
ATCTAACTAGATATTTAATGCATGGGATGATACATCCTAATCTTATAAGCGTAAAGGAT  
GGGGTCCCCCTTATTGGATTATTAAACGGGTGATATAGGTATTAAATTTAAACTATATTCCA  
CCATGAATATAAATGGGCTACGGTATGGAGATATTACGTTATCTTCATACGATATGAGTA  
ATAAATTAGTCTCTATTATTAAATACACCCATATATGAGTTAATACCGTTTACTACATGTT  
GTTCACTCAATGAATATTATTCAAAAATTGTGATTTTAATAAATGTTATTTTAGAATATA  
TGATATCTATTATATATAGAAATTTGATCGTAAAGATTTAATAACATTAAAGAAAT  
TTATTTCAAAAGTCGTAATACTGTACTAGAAATCATCAGGCATATATTTTGTTCAGATGC  
GTGTACATGAACAAATTGAAATGGAATAGATGAGCTCATTATTAAATGGATCTATGCCCTG  
TACAGCTTATGCATTTACTTCTAAAGGTAGCTACCATATAATTAGAGGAAATCAAAGAAA  
..... LysGluI  
TATAACGTAATTTTCTTTTAAATAAATAAAATACTTTTTTTAAACAAGGGTGCT  
le---  
ACCTTGTCTAATTGTATCTTGTATTTTGGATCTGATGCAAGATTATTAATAATCGTATG  
AAAAGTAGATAGATAGTTTATATCGTTACTGGACATGATATATGTTAGTTAATTCT  
TCTTTGGCATGAATTCTACACGTCGGANAAGGTAATGTATCTATATAATGCTATAAAGCTT





|           |                                                                 |    |     |     |      |
|-----------|-----------------------------------------------------------------|----|-----|-----|------|
|           | 80                                                              | 90 | 100 | 110 | 120  |
|           | ★                                                               | ★  | ★   | ★   | ★    |
| (A) VV    | DIVKALQNSCRVDEYLKAVSLYHKNSLMVSGPNVVK - LMIEYNLLTHSDLEWLINENVVKA |    |     |     |      |
| or f O1L  | :                                                               | :  | :   | :   | :    |
| (B) SPV   | DLINVIKSSVIDTRLHQSVLKHRRALIDYGQDDIITLMIINKLLSIDDISYILDKKIIHV    |    |     |     |      |
| AccI-ClaI |                                                                 |    |     |     |      |
|           |                                                                 |    |     |     | ClaI |

### FIGURE 3C

(C) W of O1L  
VLNDQYAKIVIFFNTIIIEYIIATIIYYRLTVLNNTNVKHFVSKVLHTVMEA  
570 580 590 600 610

(D) SPV EcoRV-EcoRI SLNEYYSKIVILINVILEYMSIIILYRILIVKRFNNIKEFISKVVNTVLÉS

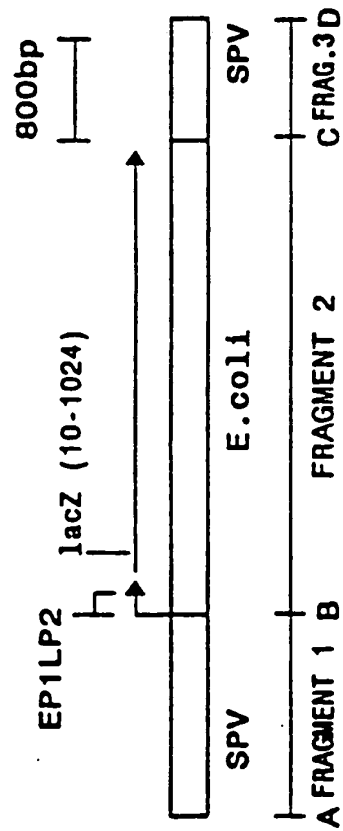
**EcoRV**

|                     | 620        | 630         | 640          | 650             | 660   | TERM                   |
|---------------------|------------|-------------|--------------|-----------------|-------|------------------------|
| (C) W or O1L        | CGVLF      | SYIKVN      | DKIEHE       | LEEMVD          | KGTVP | SYLHLSINVISIILDDINGTR. |
|                     | :          | :           | :            | :               | :     |                        |
| (D) SPV EcoRV-EcoRI | SGIYFCQMRV | HEQIELEIDEL | INGSMPVQLMHL | LKVATIILEEIKEI- |       |                        |
|                     | :          | :           | :            | :               | :     |                        |
|                     |            |             |              |                 |       | → EcoRI                |

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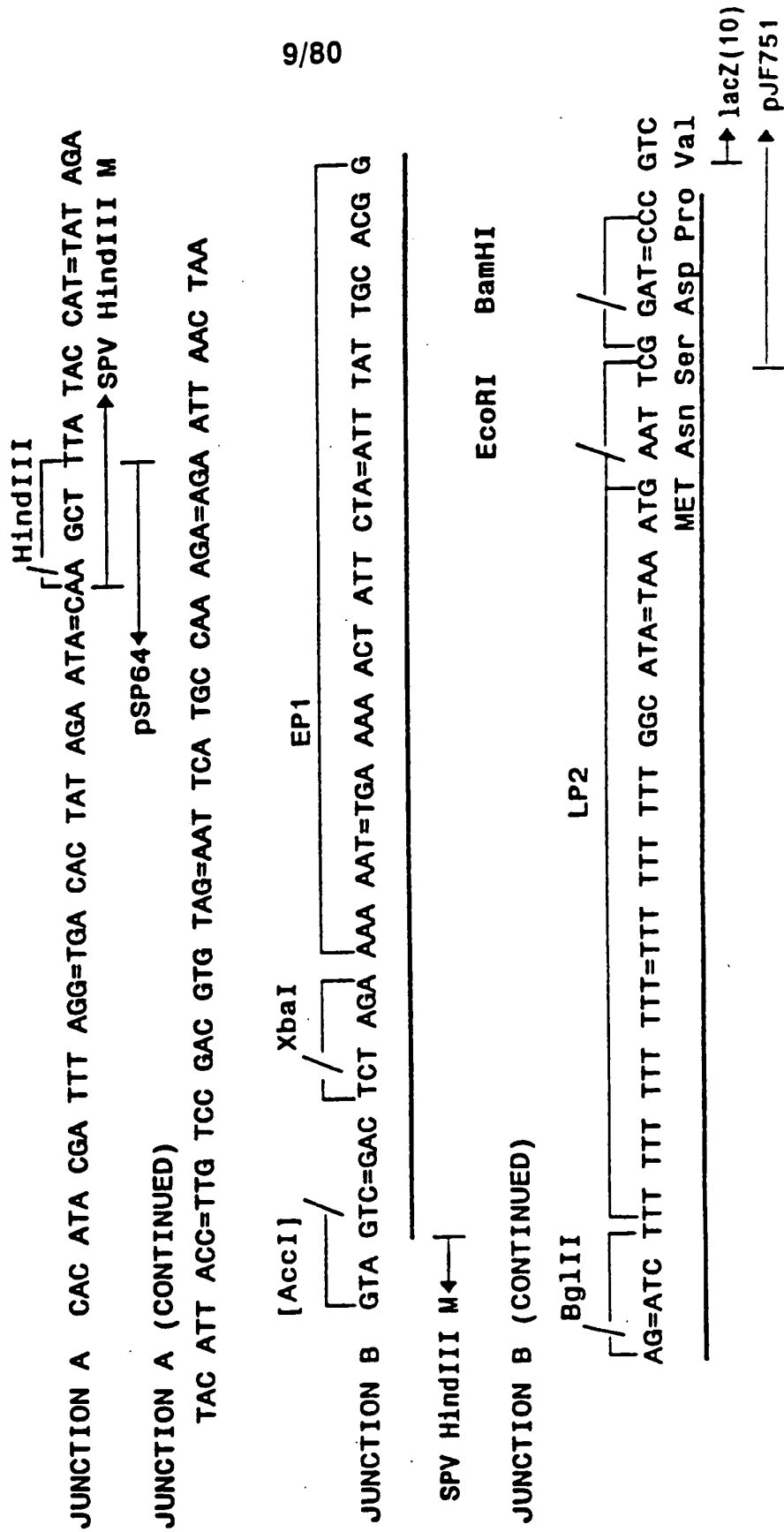
FIGURE 4A

|           |
|-----------|
| FIGURE 4A |
| FIGURE 4B |
| FIGURE 4C |



| DNA        | ORIGIN      | SLICES         | SIZE     |
|------------|-------------|----------------|----------|
| VECTOR     | pSP64       | HindIII-BamHI  | ~2972 BP |
| FRAGMENT 1 | SPV HindIII | M HindIII-AccI | ~2149 BP |
| FRAGMENT 2 | pJF751      | BamHI-PvuII    | ~3002 BP |
| FRAGMENT 3 | SPV HindIII | M AccI-BglII   | ~1484 BP |

FIGURE 4B



**FIGURE 4C**

JUNCTION C AGC CCG TCA GTA=TCG GCG GAA ATC CAG=CTG AGC GCC GGT CGC=TAC CAT TAC CAG  
Ser Pro Ser Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln

JUNCTION C (CONTINUED)

TTG=GTC TGG TGT CAA AAA=GAT CCA TAA TTA ATT=AAC CCG GGT CGA'AGA=C  
Leu Val Trp Cys Gln Lys Asp Pro ---

SPV  
HindIII M

|   | [BglII] | SmaI | SacI | EcoRI |
|---|---------|------|------|-------|
| + | +       | +    | +    | +     |
| - | -       | -    | -    | -     |

JUNCTION D AGA TCC CCG GGC GAG=CTC GAA TTC GTA ATC=ATG GTC ATA GTT=TCC

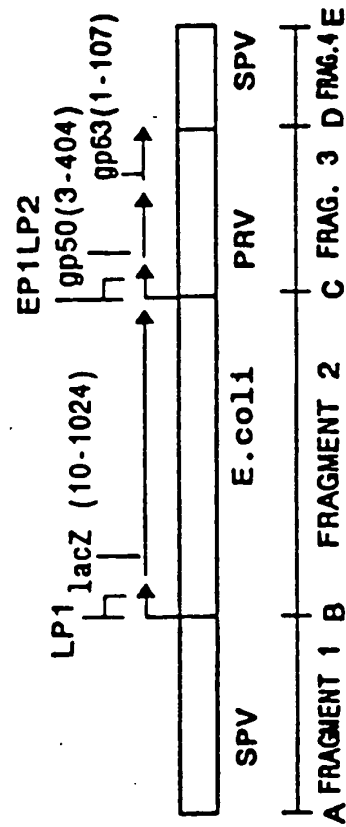
↑ pSP64

SPV Hindi M →

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FIGURE 5A

|           |
|-----------|
| FIGURE 5A |
| FIGURE 5B |
| FIGURE 5C |
| FIGURE 5D |

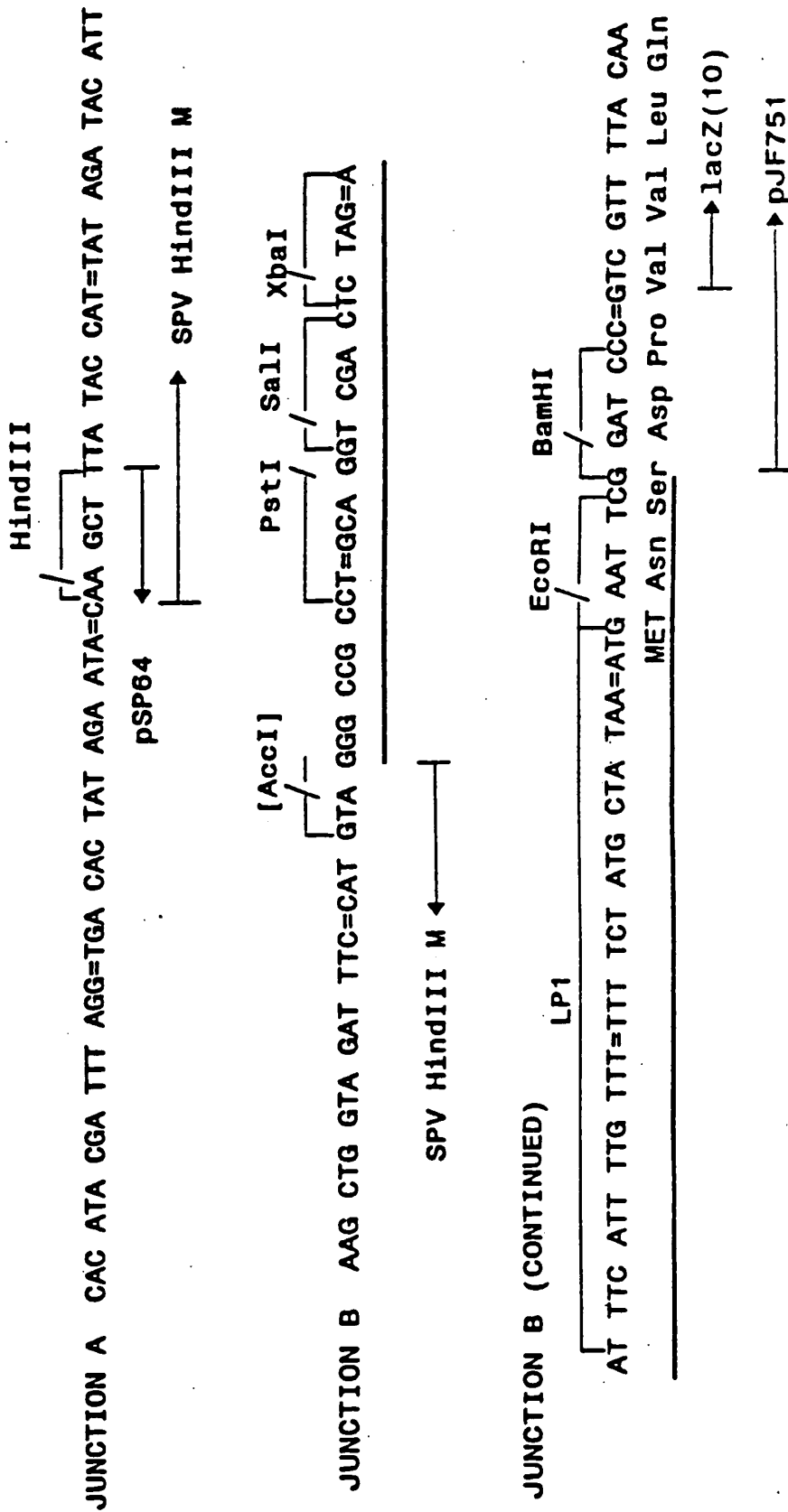


| DNA        | ORIGIN       | SITES          | SIZE     |
|------------|--------------|----------------|----------|
| VECTOR     | pSP64        | HindIII-BamHI  | ~2972 BP |
| FRAGMENT 1 | SPV HindIII  | M HindIII-AccI | ~2149 BP |
| FRAGMENT 2 | pJF751       | BamHI-PvuII    | ~3002 BP |
| FRAGMENT 3 | PRV BamHI #7 | ECORI*-StuI    | ~1558 BP |
| FRAGMENT 4 | SPV HindIII  | M AccI-BglII   | ~1484 BP |

\* INTRODUCED VIA CLONING

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FIGURE 5B





**FIGURE 5C**  
**PvuII**

JUNCTION C    GTA TCG GCG GAA ATC CAG CTG AGC=GCC GGT CGC TAC CAT=TAC CAG TTG GTC TGG=TGT  
Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys

pJF7514

CAA=AAA GAT CCA TAA TTA=ATT AAC CCG GCC GCC=TGC AGG TGG ACT

**lacZ (1024)**

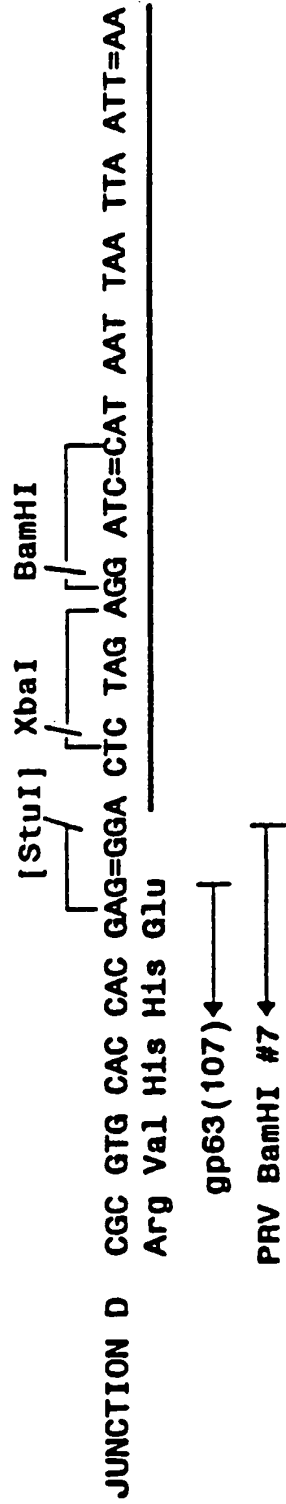
CT AGA AAA AAT TGA=AAA ACT ATT CTA ATT=TAT TGC ACG GAG ATC=T

XbaI EP1

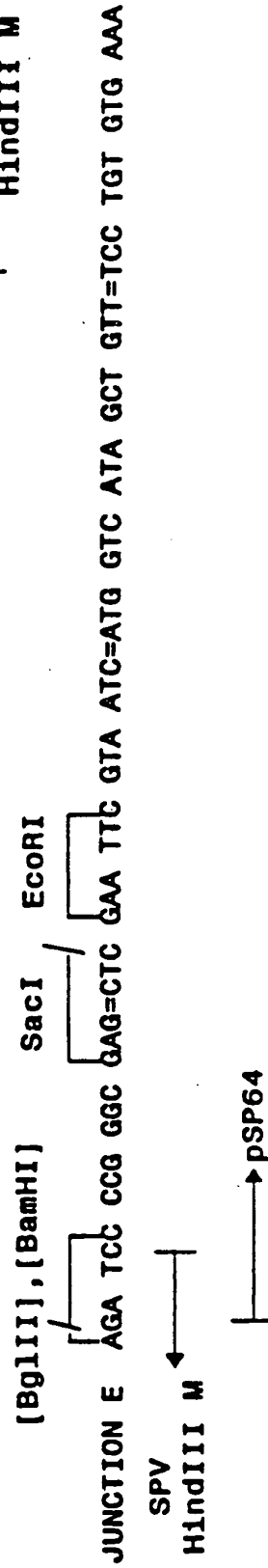
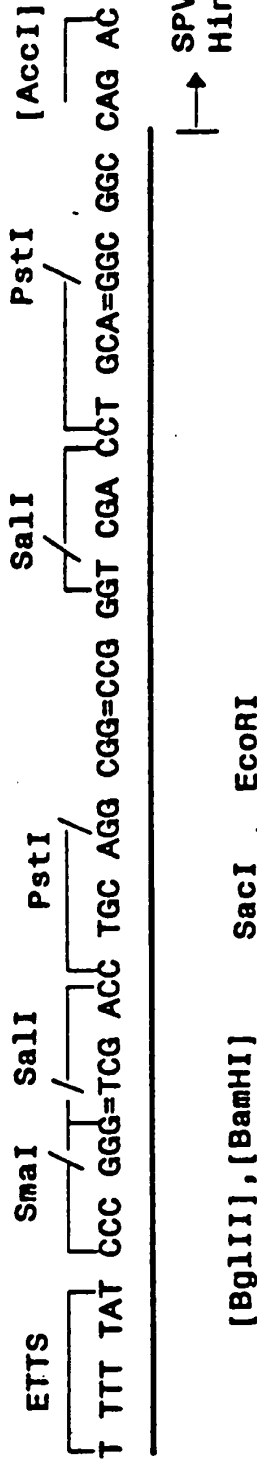
LP2  
 TTT TTT TTT TTT TTT GGC ATA TAA=ATG AAT TCG CTC GCA=GCG CTA TTG GCG GCG  
 MET Asn Ser Leu Ala Ala Leu Leu Ala Ala  
 → gp50(3)  
 → PRV BamHI #7

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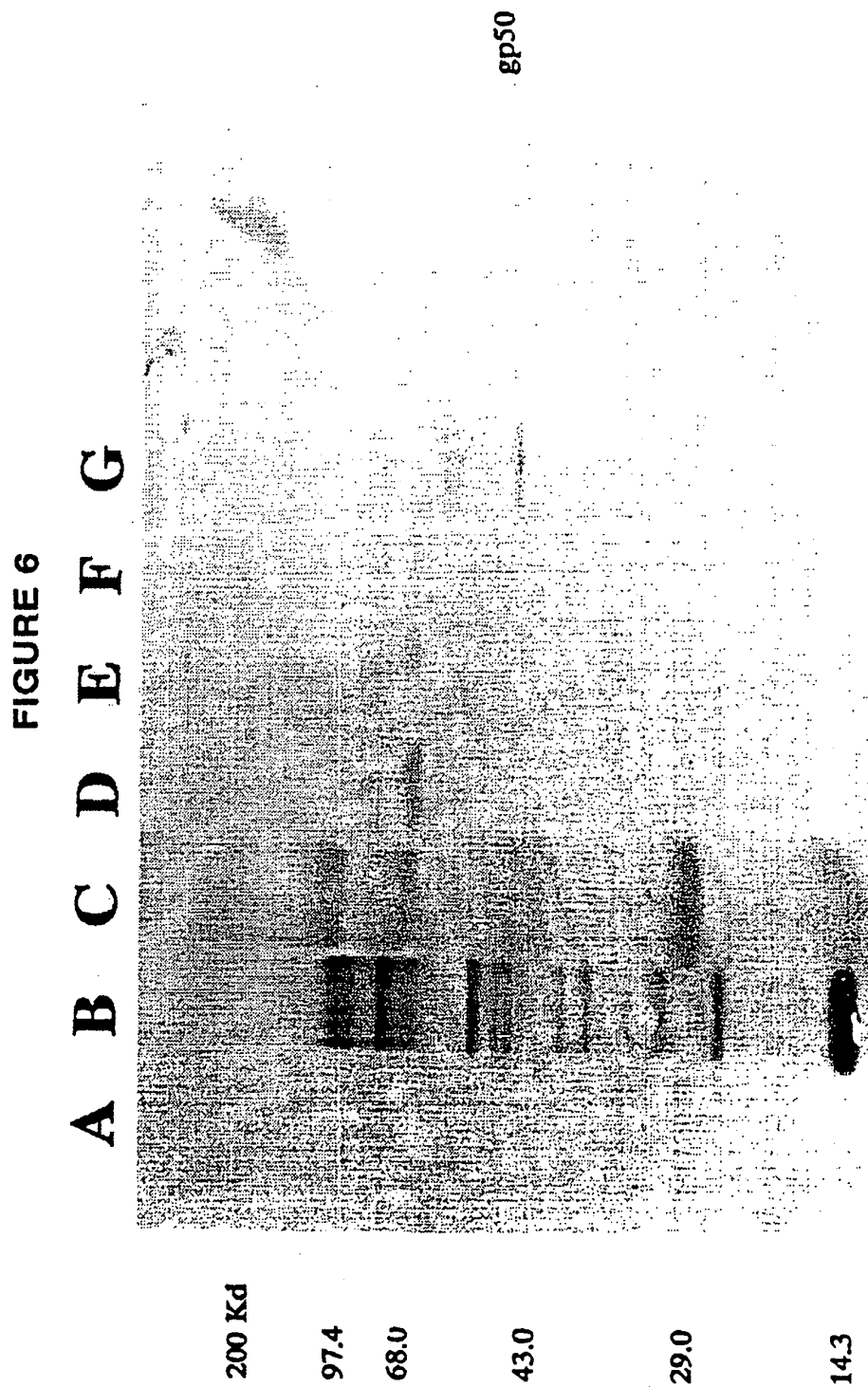
FIGURE 5D



JUNCTION D (CONTINUED)



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## FIGURE 7

ACGGGTAGAACGGTAAGAGAGGCGCGCCCTCAATTGCGAGCCAGACTTCACAACCTCCGT

AvaII

r/—

TCTACCGCTTCACCGACAACAGTCCTCAATCATGGACCGCGCCGTTAGCCAAGTTGCGTT

MetAspGly.....

AGAGAATGATGAAAGAGAGGCCAAAAATACATGGCGCTTGATATCCGGATTGCAATCTT  
 ATTCTTAACAGTAGTGACCTTGGCTATATCTGTAGCCTCCCTTTTATATAGCATGGGGGC  
 TAGCACACCTAGCGATCTTGTAGGCATACCGACTAGGATTTCCAGGGCAGAAGAAAAGAT  
 TACATCTACACTTGGTTCCAATCAAGATGTAGTAGATAGGATATATAAGCAAGTGGCCCT  
 TGAGTCTCCATTGGCATTGTTAAATACTGAGACCACAATTATGAACGCAATAACATCTCT  
 CTCTTATCAGATTAATGGAGCTGCAACAACAGCGGGTGGGGGGCACCTATTCATGACCC  
 AGATTATATAGGGGGGATAGGCAAAGAACTCATTGTAGATGATGCTAGTGATGTACATC  
 ATTCTATCCCTCTGCATTTCAAGAACATCTGAATTTTATCCCGGCGCCTACTACAGGATC  
 AGGTTGCACTCGAATACCCCTCATTTGACATGAGTGCTACCCATTACTGCTACACCCATAA  
 TGTAATATTGTCTGGATGCAGAGATCACTCACACTCACATCAGTATTTAGCACTTGGTGT  
 GCTCCGGACATCTGCAACAGGGAGGGTATTCTTTTCTACTCTGCGTTCCATCAACCTGGA  
 CGACACCCAAAATCGGAAGTCTTGCACTGTGAGTGCAACTCCCCTGGGTTGTGATATGCT  
 GTGCTCGAAAGCCACGGAGACAGAGGAAGAAGATTATAACTCAGCTGTCCCTACGCGGAT  
 GGTACATGGGAGGTTAGGGTTCCAGCGCCAATATCAGAAAAGGACCTAGATGTCACAAC  
 ATTATTCGGGGAAGTGGGTGGCCAACTACCCAGGAGTAGGGGGTGGATCTTTTATTGACAG  
 CCGCGTGTGGTTCTCAGTCTACGGAGGGTTAAACCCTAATACACCCAGTGACACTGTACA  
 GGAAGGCAAAATATGTGATATACAAGCCATACAATGACACATGCCCAGATGAGCAAGACTA  
 CCAGATTGCAATGCGCAAGTCTTCGTATAAGCCTGGACGGTTTGGTGGGAAACGCATACA  
 GCAGGCTATCTTATCTATCAAAGTGTCAACATCCTTAGGGCAAGACCCGGTACTGACTGT  
 ACCGCCCAACACAGTCACACTCATGGGGCCGAAGGCAGAATTCTCACAGTAGGGACATC  
 CCATTTCTTGATCAGCGAGGGTCATCATACTTCTCTCCCGCGTTATTATATCCTATGAC  
 AGTCAGCAACAAAACAGCCACTCTTCATAGTCCTTATACATTCAATGCCTTCACTCGGCC  
 AGGTAGTATCCCTTGCCAGGCTTCAGCAAGATGCCCCAACTCATGTGTTACTGGAGTCTA  
 TACAGATCCATATCCCCTAATCTTCTATAGAAACCACACCTTGGGAGGGGTATTCCGGAC  
 AATGCTTGATGGTGAACAAGCAAGACTTAACCTGCGTCTGCAGTATTCGATAGCACATC  
 CCGCAGTCGCATAACTCGAGTCAGTTCAAGCAGCATCAAAGCAGCATACACAACATCAAC  
 TTGTTTTAAAGTGGTCAAGACCAATAAGACCTATTGTCTCAGCATTGCTGAAATATCTAA  
 TACTCTCTTCGGAGAATTCAGAATCGTCCCGTTACTAGTTGAGATCCTCAAAGATGACGG  
 GGTAGAGAAGCCAGGTCTGGCTAGTTCAGTCAACTATGAAAGAGTTGGAAAGATGGCAT  
 .....ArgSerGly---

NaeI

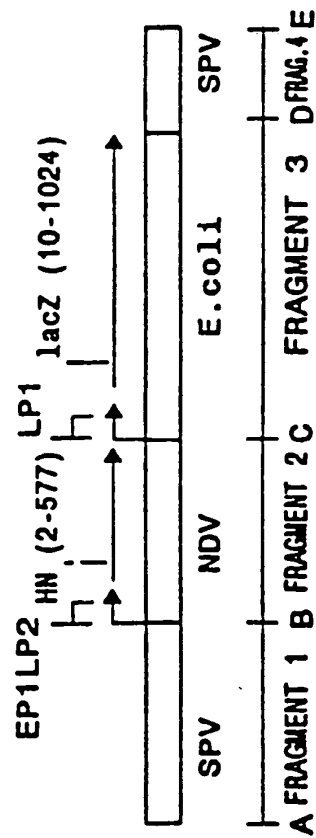
—/—

TGTATCACCTATCTTCTGCGACATCAAGAATCAAACCGAATGCCGGC

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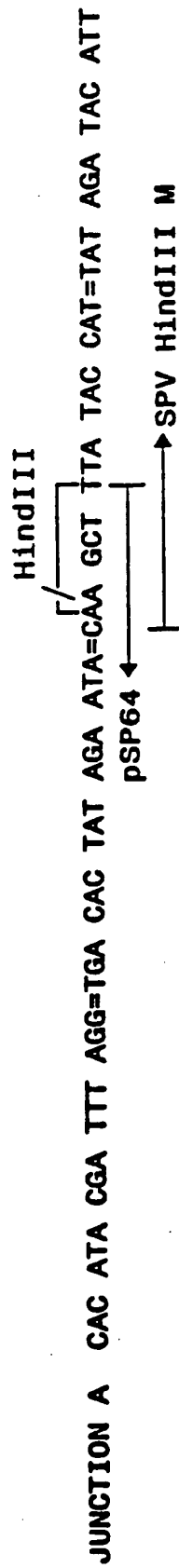
FIGURE 8A

|           |
|-----------|
| FIGURE 8A |
| FIGURE 8B |
| FIGURE 8C |
| FIGURE 8D |



| DNA        | ORIGIN      | SLICES         | SIZE     |
|------------|-------------|----------------|----------|
| VECTOR     | pSP64       | HindIII-BamHI  | ~2972 BP |
| FRAGMENT 1 | SPV HindIII | M HindIII-AccI | ~2149 BP |
| FRAGMENT 2 | MDV HN cDNA | AvaII-NaeI     | ~1810 BP |
| FRAGMENT 3 | pJF751      | BamHI-PvuII    | ~3002 BP |
| FRAGMENT 4 | SPV HindIII | M AccI-BglII   | ~1484 BP |

FIGURE 8B



SPV HindIII M ←

JUNCTION B (CONTINUED)

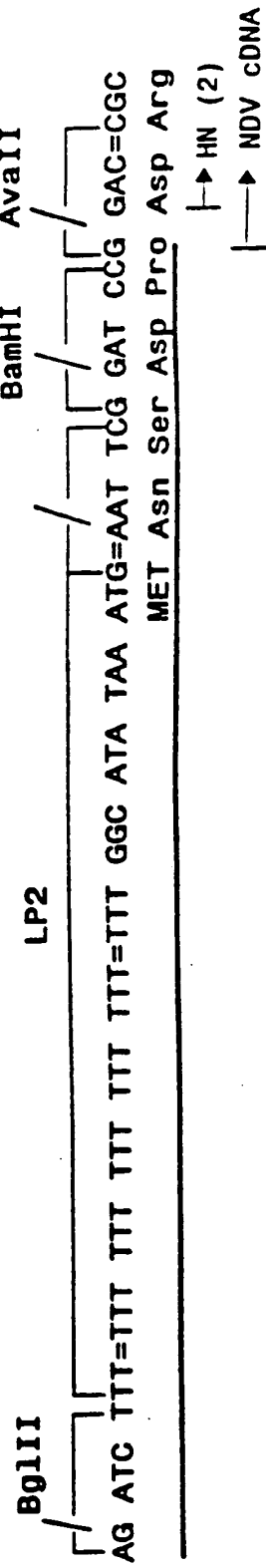
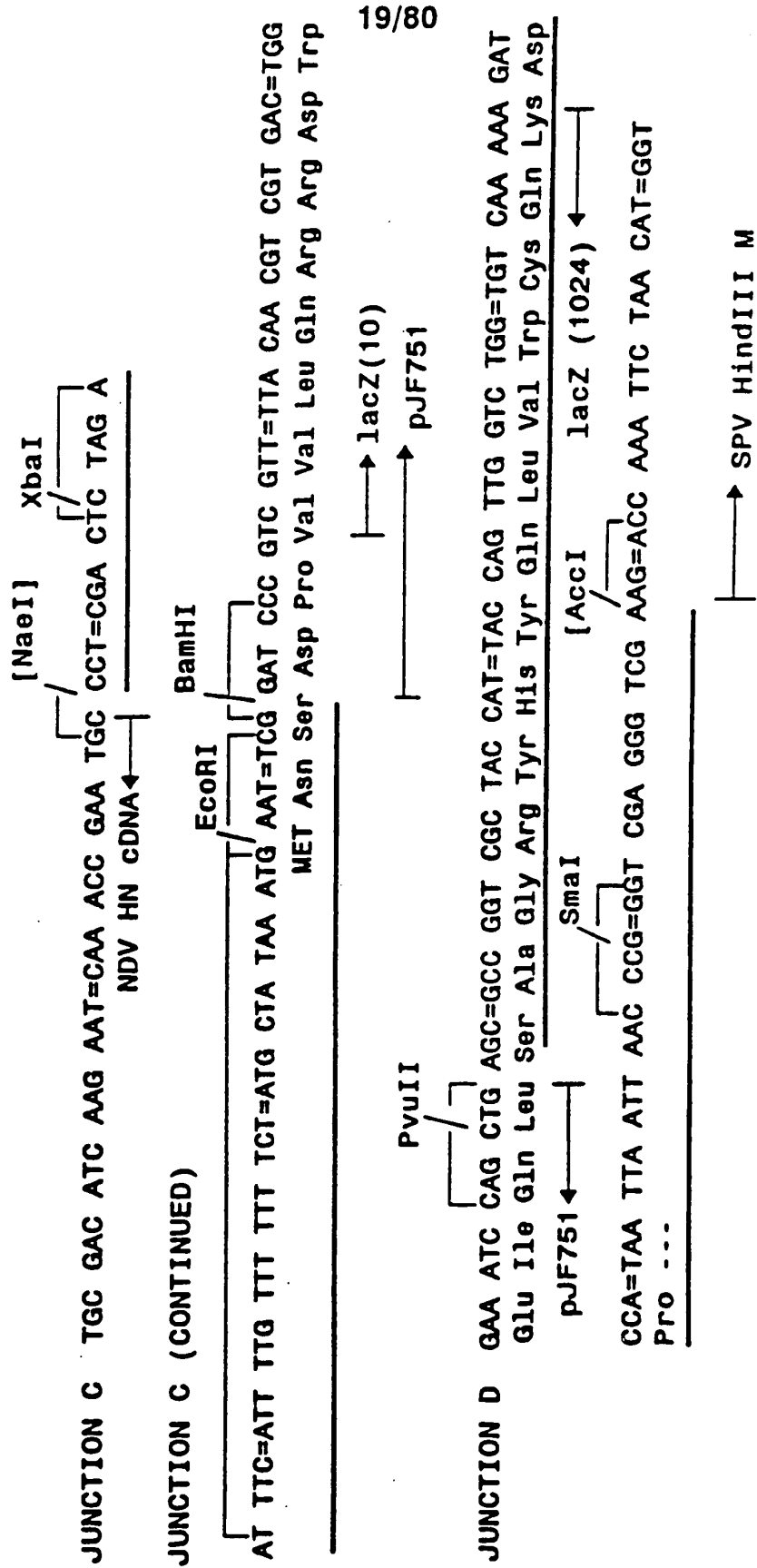
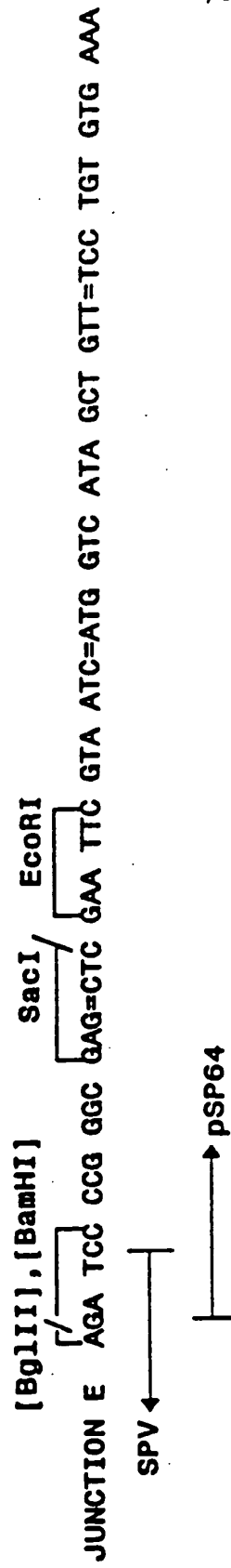


FIGURE 8C



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FIGURE 8D





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FIGURE 9A  
FIGURE 9B  
FIGURE 9C

FIGURE 9A

| DNA        | Origin        | Sites           | Size     |
|------------|---------------|-----------------|----------|
| Vector     | pSP64         | Bam HI—Hind III | ~2972 BP |
| Fragment 1 | SPV HindIII G | Hind III—Nde I  | ~ 670 BP |
| Fragment 2 | pJF751        | Bam HI—Pvu II   | ~3010 BP |
| Fragment 3 | SPV HindIII G | Nde I—Bam HI    | ~1069 BP |

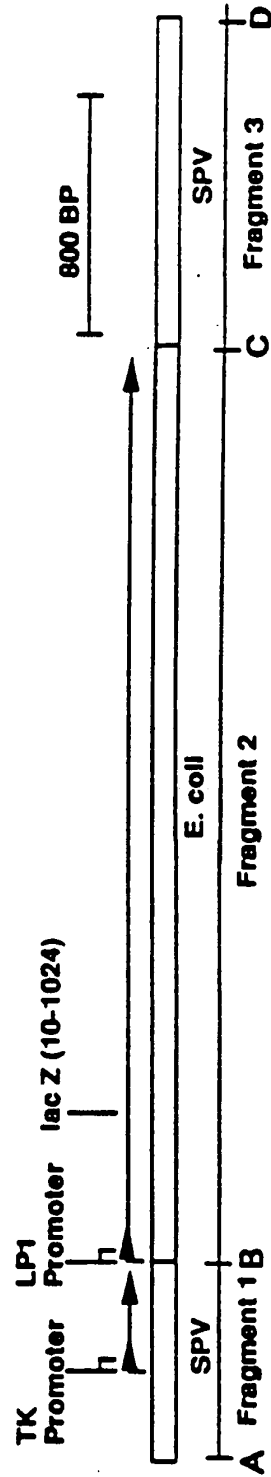


FIGURE 9B

JUNCTION A

CAC ATA CGA TTT AGG TGA CAC TAT AGA ATA CAA GCT TTG AGT CTA TTG GTT ATT TAT ACG  
 pSP64  
 Hind III  
 SPV Hind III G

JUNCTION B

TGA ATA TAT AGC AAA TAA AGG AAA AAT TGT TAT CGT TGC TGC ATT AGA TGG AAC ATA GG  
 [Nde I]  
 SPV TK

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JUNCTION B (CONT.)

LP1  
 T CGA CTC TAG AAT TTC ATT TTG TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA  
 MET Asn Ser Asp Pro Val Val Leu  
 EcoRI BamHI  
 E. coli Lac Z (10)  
 pJF 751

FIGURE 9C

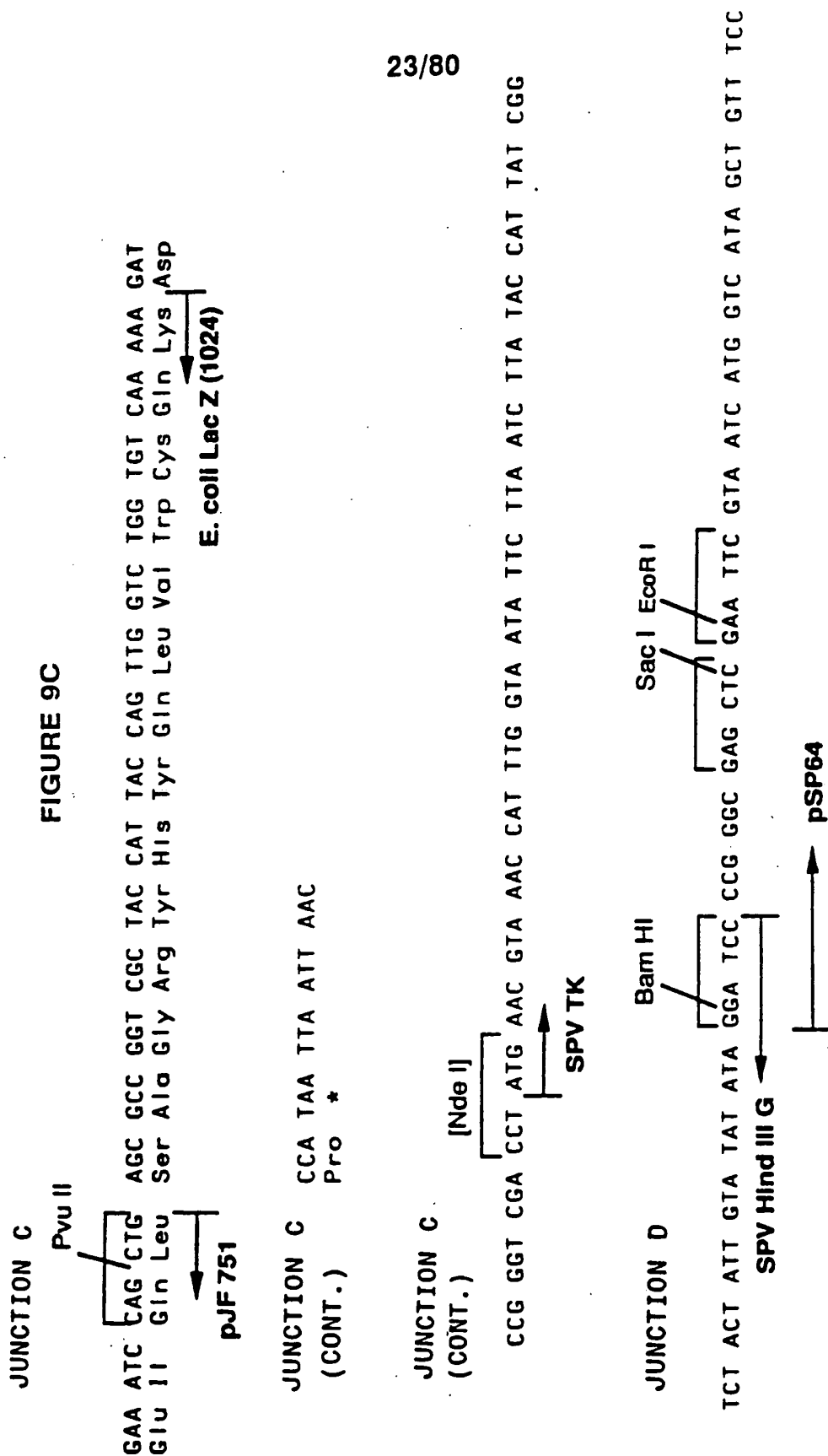


FIGURE 10A  
FIGURE 10B  
FIGURE 10C  
FIGURE 10D

FIGURE 10A

| DNA        | Origin          | Sites           | Size     |
|------------|-----------------|-----------------|----------|
| Vector     | pSP84           | Hind III—Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M   | Bgl II—Acc I    | ~1484 BP |
| Fragment 2 | pJF751          | Bam HI—Pvu II   | ~3002 BP |
| Fragment 3 | PRV BamHI 2 & 9 | Nco I—Nco I     | ~2378 BP |
| Fragment 4 | SPV HindIII M   | Acc I—Hind III  | ~2149 BP |

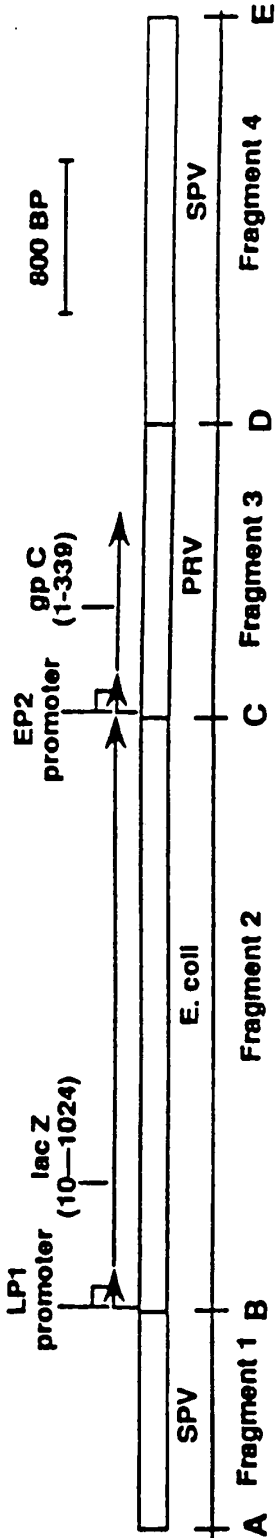
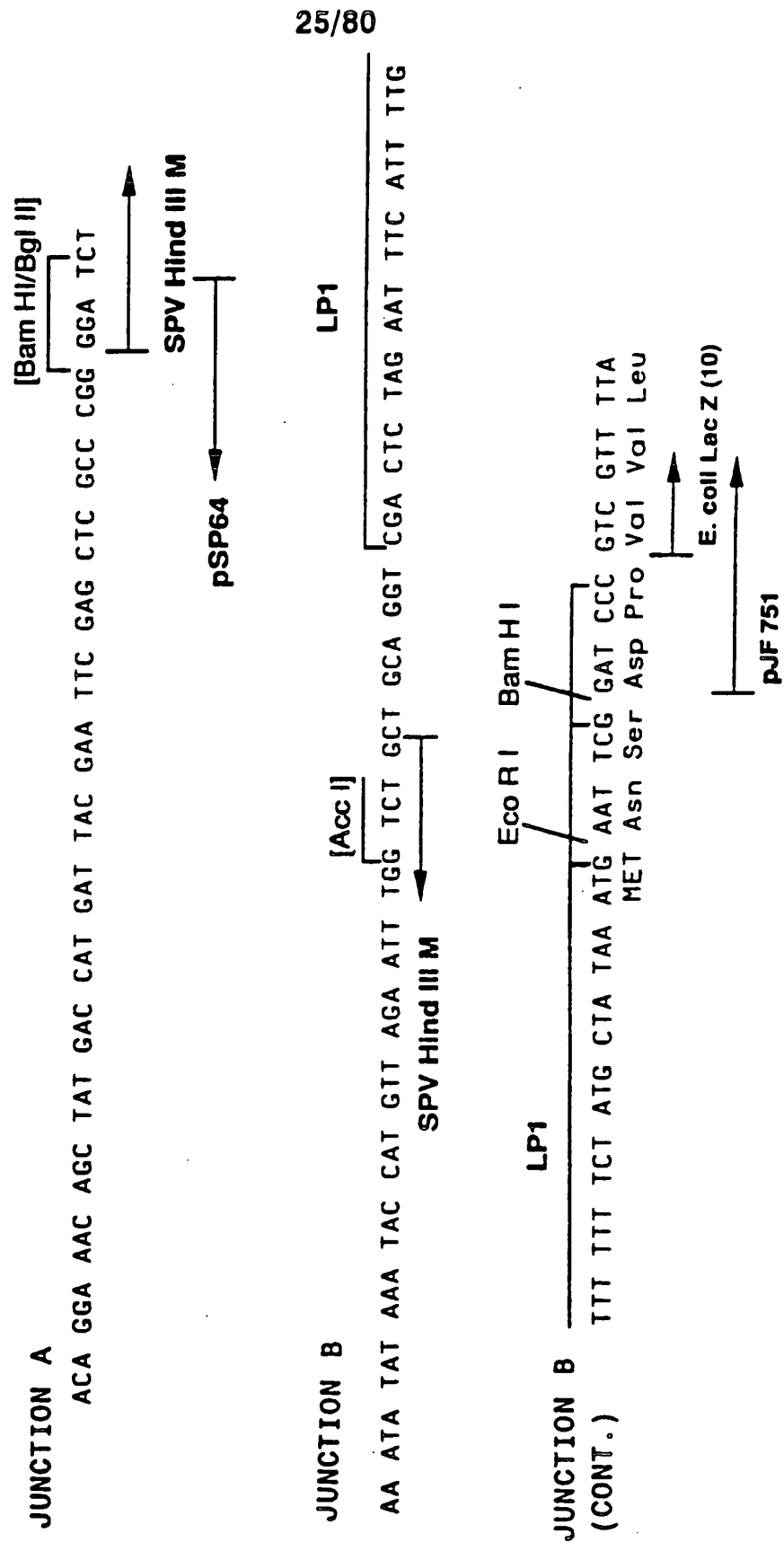
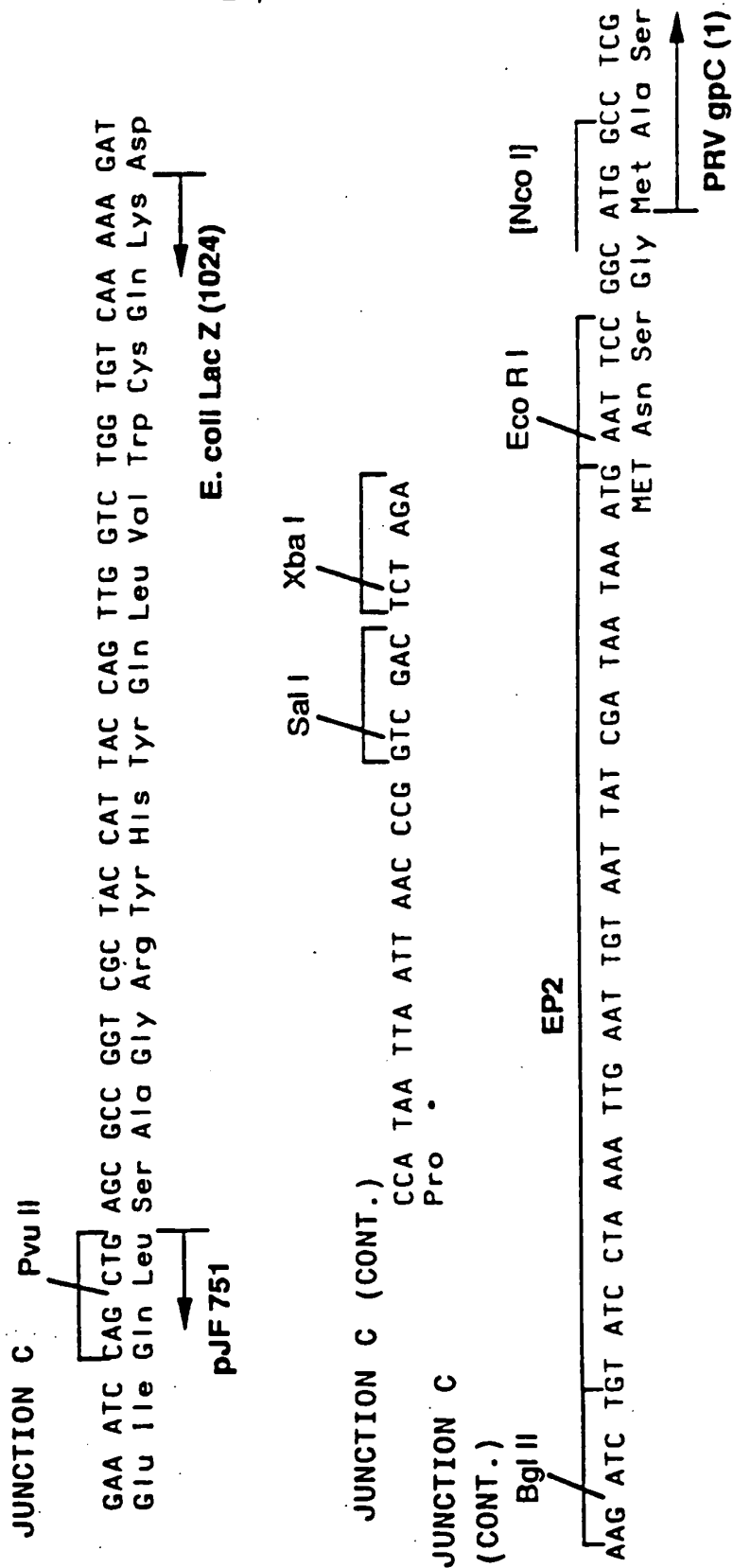


FIGURE 10B



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FIGURE 10C



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FIGURE 10D

JUNCTION D

$\overline{\text{[Nco I]}}$   
 C CAT GCT CTA GAG GAT CCC CGG GCG AGC TCG AAT TCG GAT CCA TAA TTA ATT  
 PRV Bam HI #9  
 EcoRI

JUNCTION D (CONT.)

AAT TAA TTT TTA TCC CGG GTC GAC CGG GTC GAC CTG CAG CCT ACA TGG AAA TCT ACC  
 $\overline{\text{[Acc I]}}$   
 SPV Hind III M

JUNCTION E

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC  
 HindIII  
 pSP64  
 SPV Hind III M

FIGURE 11A  
FIGURE 11B  
FIGURE 11C  
FIGURE 11D

FIGURE 11A

| DNA        | Origin          | Sites           | Size     |
|------------|-----------------|-----------------|----------|
| Vector     | pSP64           | Hind III—Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M   | Bgl II—Acc I    | ~1484 BP |
| Fragment 2 | pJF751          | Bam HI—Pvu II   | ~3002 BP |
| Fragment 3 | PRV BamHI 2 & 9 | Nco I—Nco I     | ~2378 BP |
| Fragment 4 | SPV HindIII M   | Acc I—Hind III  | ~2149 BP |

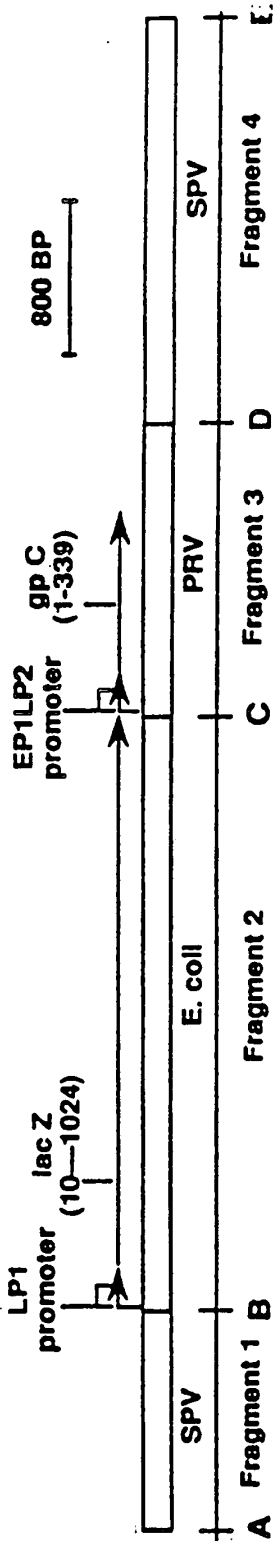




FIGURE 11B

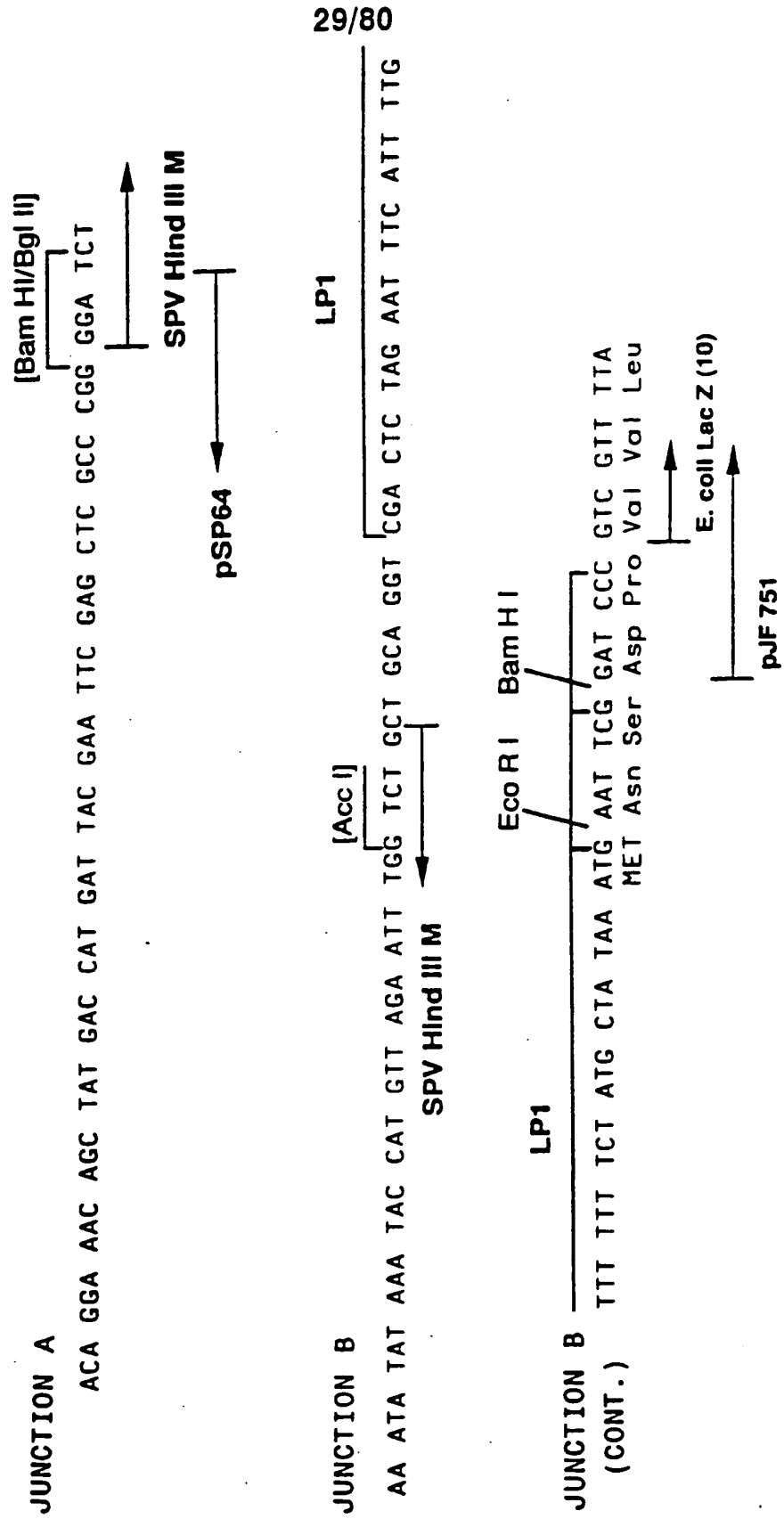
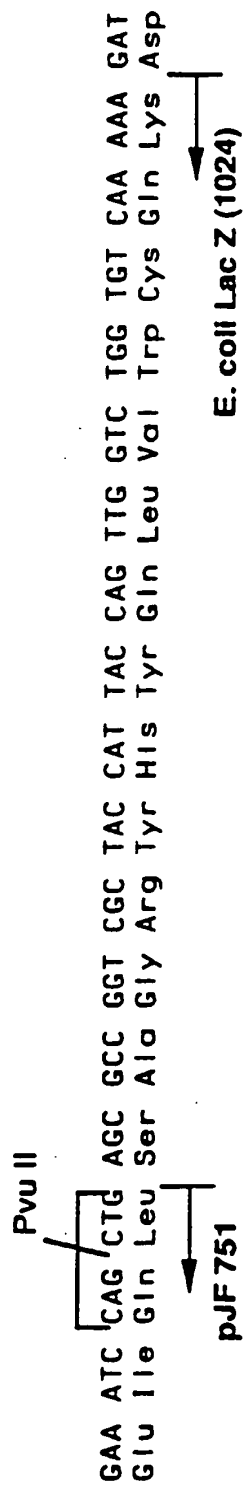


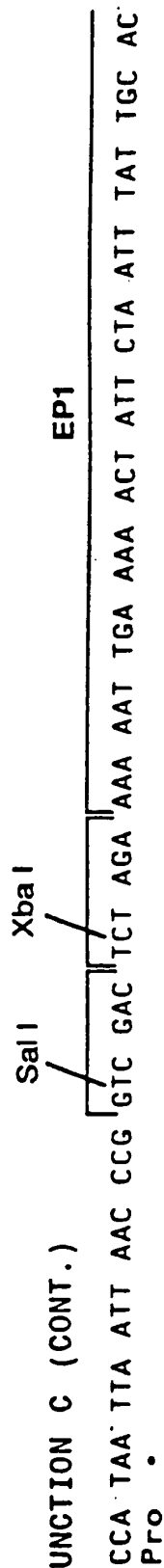
FIGURE 11C

JUNCTION C



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JUNCTION C (CONT.)



JUNCTION C (CONT.)

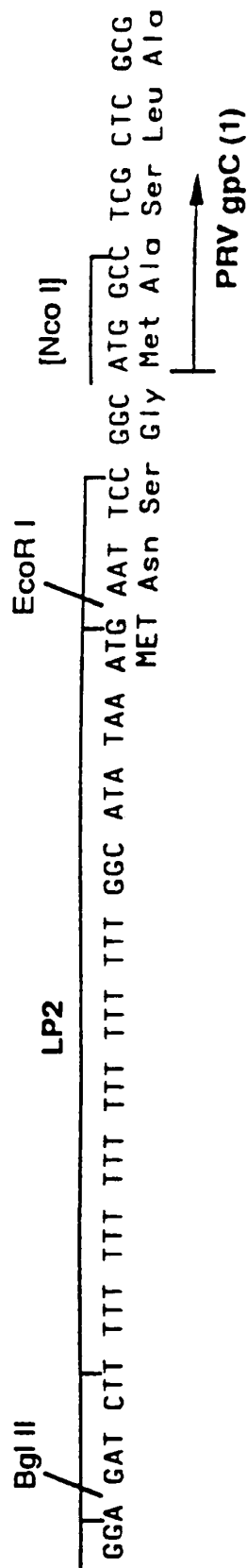


FIGURE 11D

JUNCTION D

[Nco I]  
C CAT GCT CTA GAG GAT CCC CGG GCG AGC TCG AAT TCG GAT CCA TAA TTA ATT  
PRV Bam HI #9  
EcoRI

JUNCTION D (CONT.)

31/80  
AAT TAA TTT TTA TCC CGG GTC GAC CGG GTC GAC CTG CAG CCT ACA TGG AAA TCT ACC  
[Acc I]  
SPV Hind III M

JUNCTION E

TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC  
HindIII  
pSP64  
SPV Hind III M



FIGURE 12B

JUNCTION A

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

[Bam HI/Bgl II]

SPV Hind III M

pSP64

33/80

JUNCTION B

AA ATA TAT AAA TAC CAT GTT AGA ATT TGG TCT GCT GCA GGT CGA CTC TAG AAT TTC ATT TTG

[Acc I]

SPV Hind III M

LP1

JUNCTION B  
(CONT.)

TTT TTT TCT ATG CTA TAA ATG AAT TCG GAT CCC GTC GTT TTA  
MET Asn Ser Asp Pro Val Val Leu

Eco RI Bam HI

LP1

E. coli Lac Z (10)

pJF 751



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FIGURE 12D

JUNCTION D

[Nco I]  
 C CAT GCT CTA GAG GAT CCC CGG GCG AGC TCG AAT TCG GAT CCA TAA TTA ATT  
 PRV Bam HI #9  
 EcoRI

JUNCTION D (CONT.)

AAT TAA TTT TTA TCC CGG GTC GAC CGG GTC GAC CTG CAG CCT ACA TGG AAA TCT ACC  
[Acc I]  
 SPV Hind III M

JUNCTION E

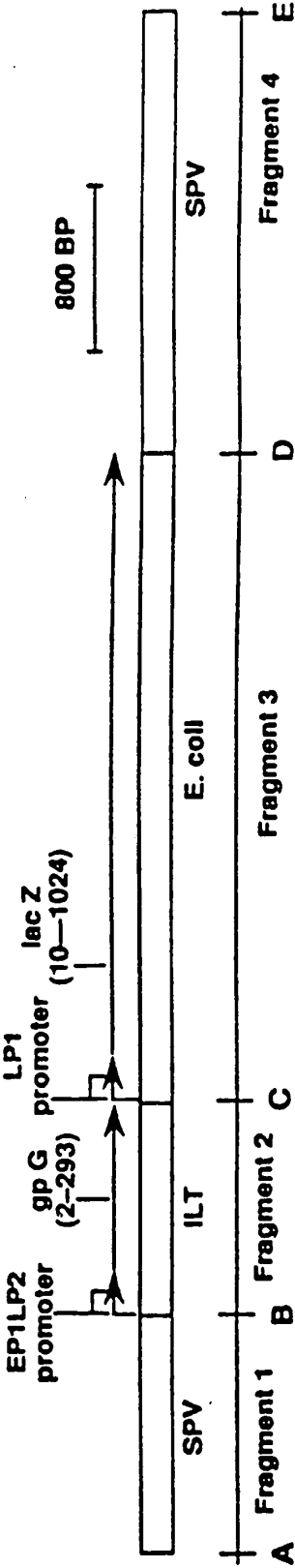
TAA TGT ATC TAT AAT GGT ATA AAG CTT GTA TTC TAT AGT GTC ACC TAA ATC  
 HindIII  
 pSP64  
 SPV Hind III M

FIGURE 13A  
FIGURE 13B  
FIGURE 13C  
FIGURE 13D

FIGURE 13A

| DNA        | Origin                | Sites           | Size     |
|------------|-----------------------|-----------------|----------|
| Vector     | pSP64                 | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M         | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | ILT Asp718I<br>5.1 kb | Eco RI†-Mbo I   | ~ 939 BP |
| Fragment 3 | pJF751                | Bam HI-Pvu II   | ~3002 BP |
| Fragment 4 | SPV HindIII M         | Acc I-Hind III  | ~2149 BP |

† Restriction site introduced by PCR cloning





[Bam HI/Bgl II]  
 3 CTC GCC CGG GGA TCT  
 SPV Hind III M  
 pSP64

[Acc I]      Pst I      Sal I      Xba I      EP1  
 GGT CTG    CTG CAG    GTC GAC    TCT AGA    AAA AAT TGA AAA ACT ATT CTA ATT TAT TGC ACG G  
 SPV Hind III M

**Bgl II**

**LP2**

Eco RI

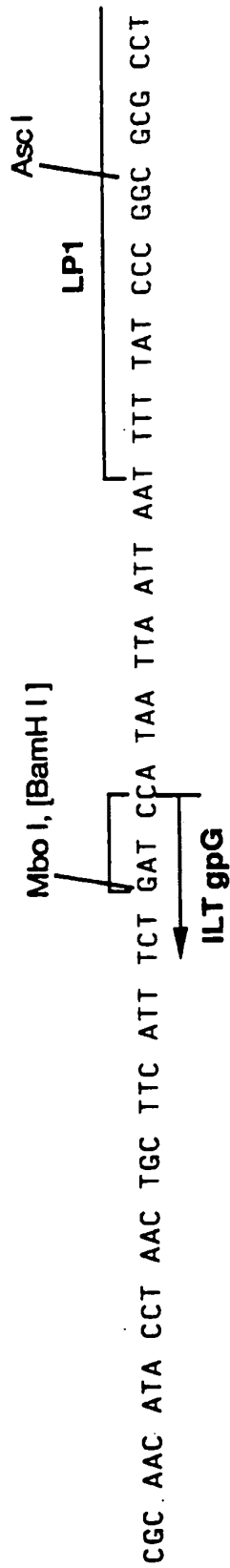
AG ATC TTT TTT TTT TTT TTT TTT GGC ATA TAA ATG AAT TTC GGC TTC AGT AAC ATA  
MET Asn Ser Gly Phe Ser Asn Ile

→ILT gp G(2)

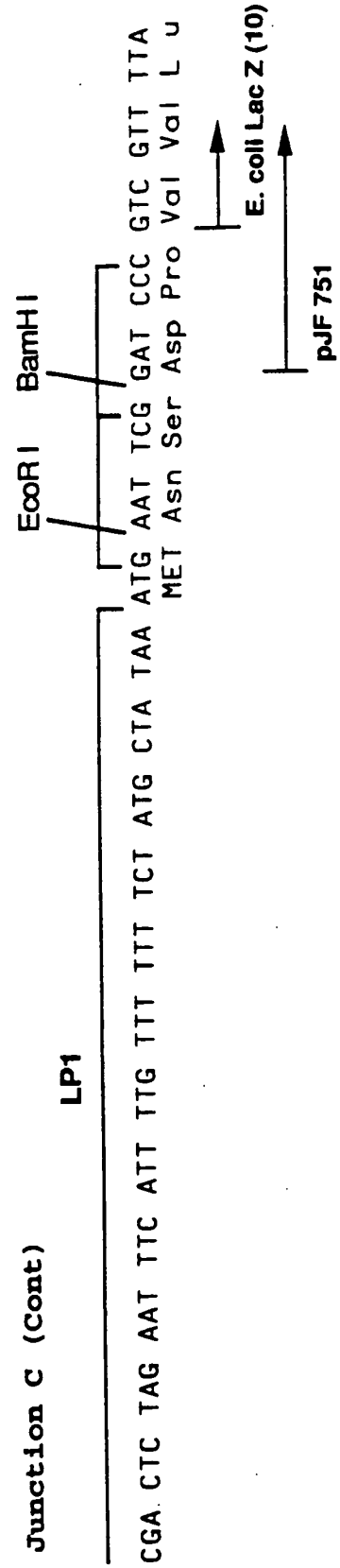
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FIGURE 13C

Junction C

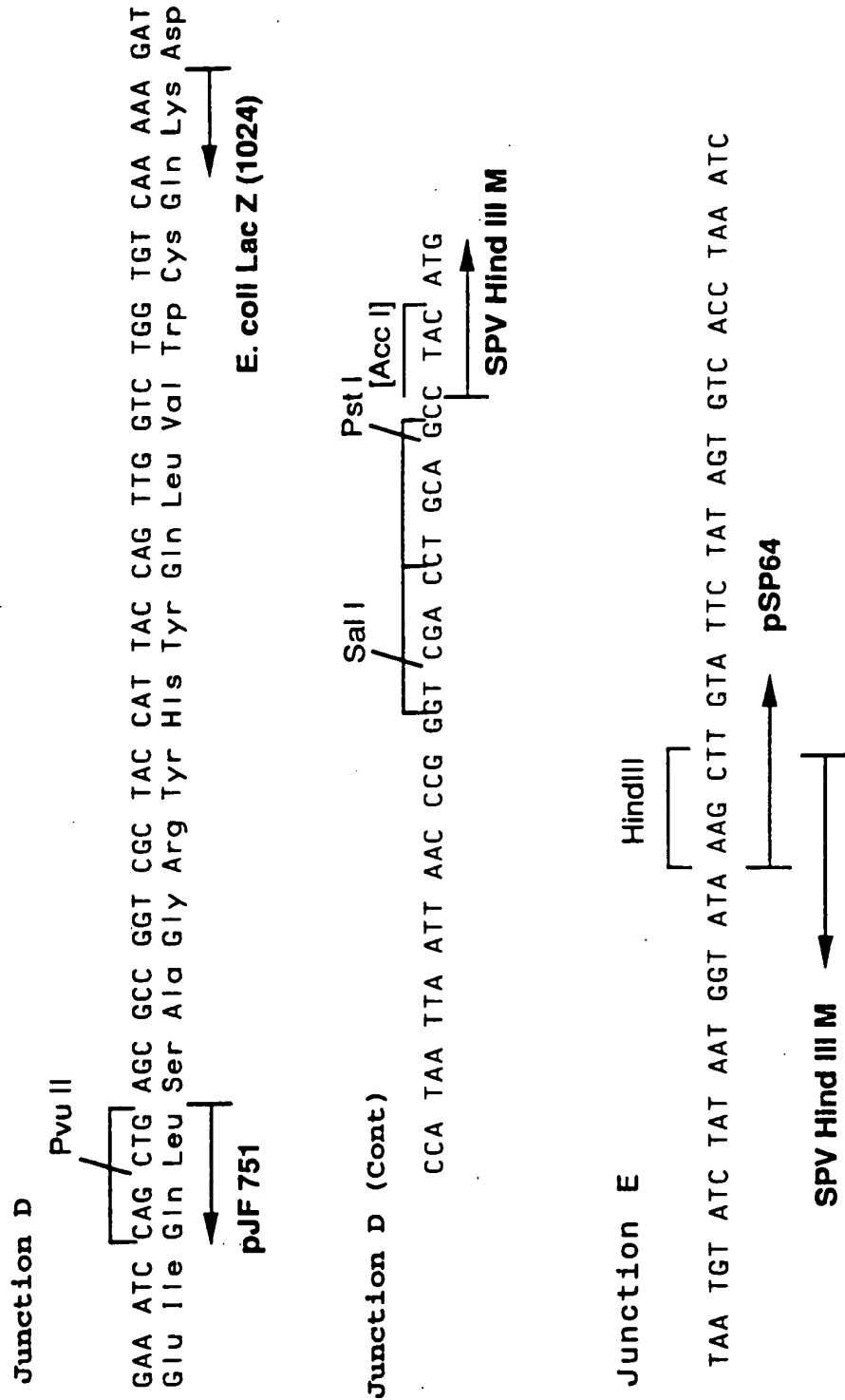


Junction C (Cont)



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FIGURE 13D

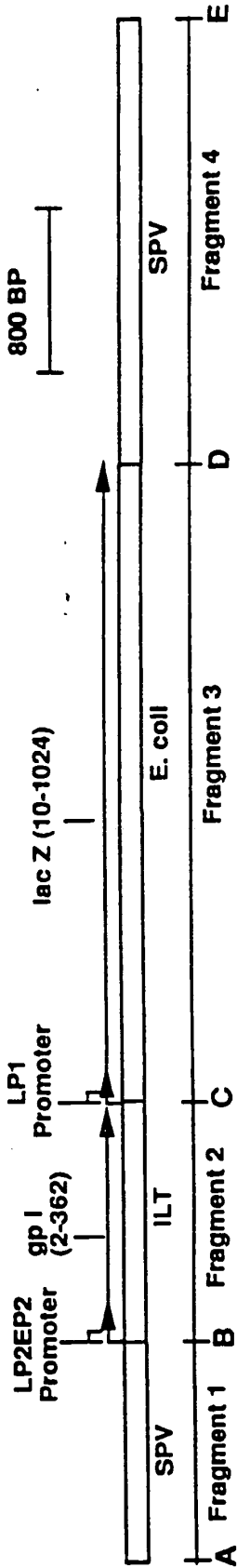


|            |
|------------|
| FIGURE 14A |
| FIGURE 14B |
| FIGURE 14C |
| FIGURE 14D |

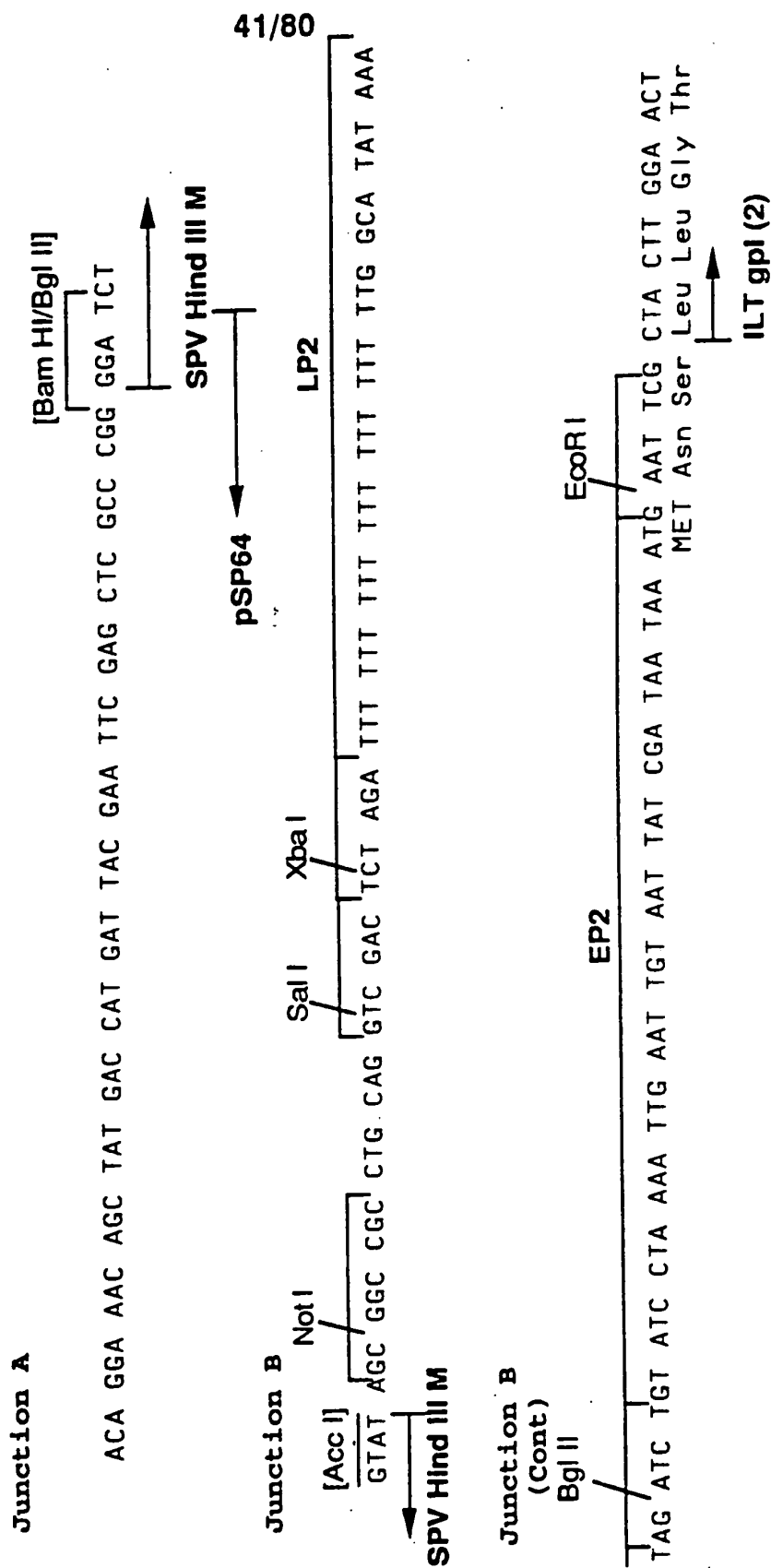
FIGURE 14A

| DNA        | Origin         | Sites                     | Size     |
|------------|----------------|---------------------------|----------|
| Vector     | pSP64          | Hind III-Bam HI           | ~2972 BP |
| Fragment 1 | SPV Hind III M | Bgl II-Acc I              | ~1484 BP |
| Fragment 2 | ILT Asp 718I   | Eco RI†-Bam HI†<br>8.0 kb | ~1090 BP |
| Fragment 3 | pJF751         | Bam HI-Pvu II             | ~3010 BP |
| Fragment 4 | SPV Hind III M | Acc I-Hind III            | ~2149 BP |

†Restriction sites introduced by PCR cloning

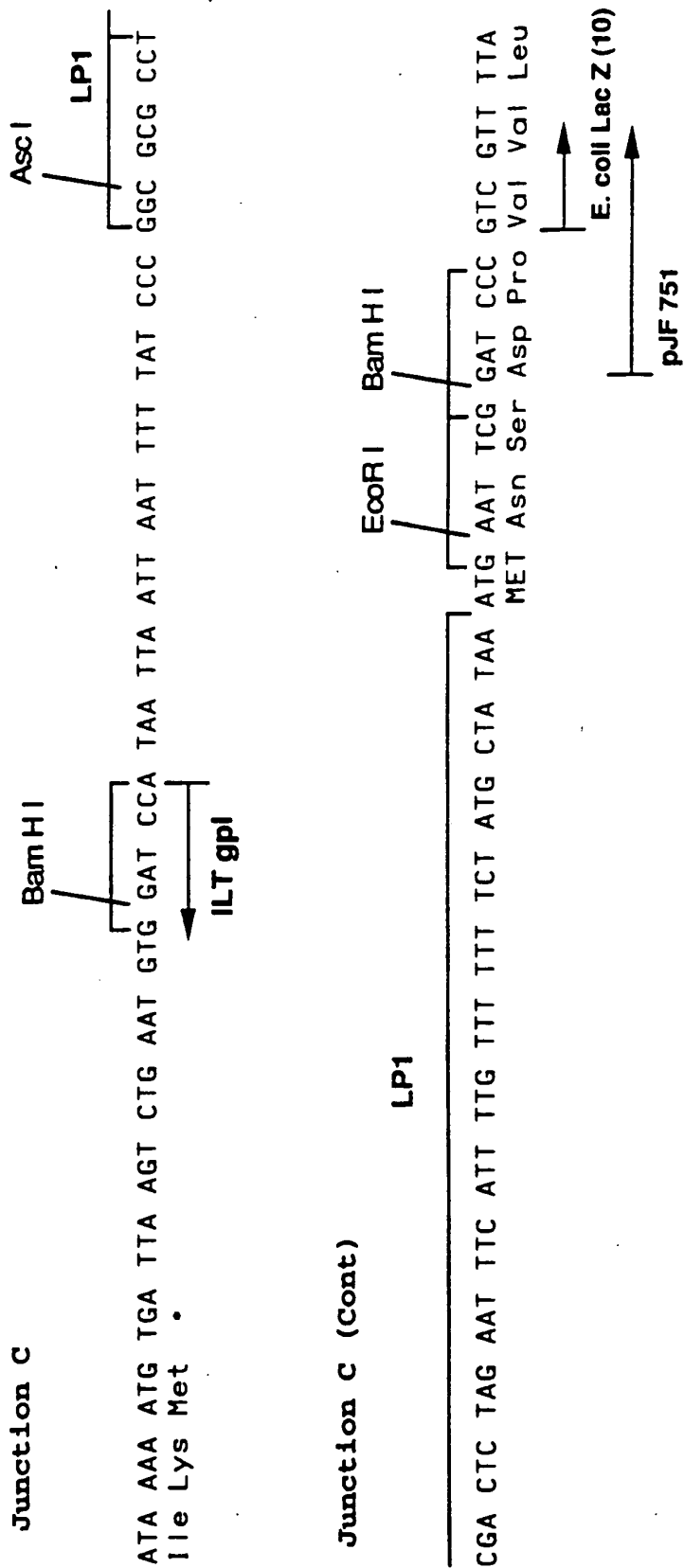


**FIGURE 14B**



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FIGURE 14C



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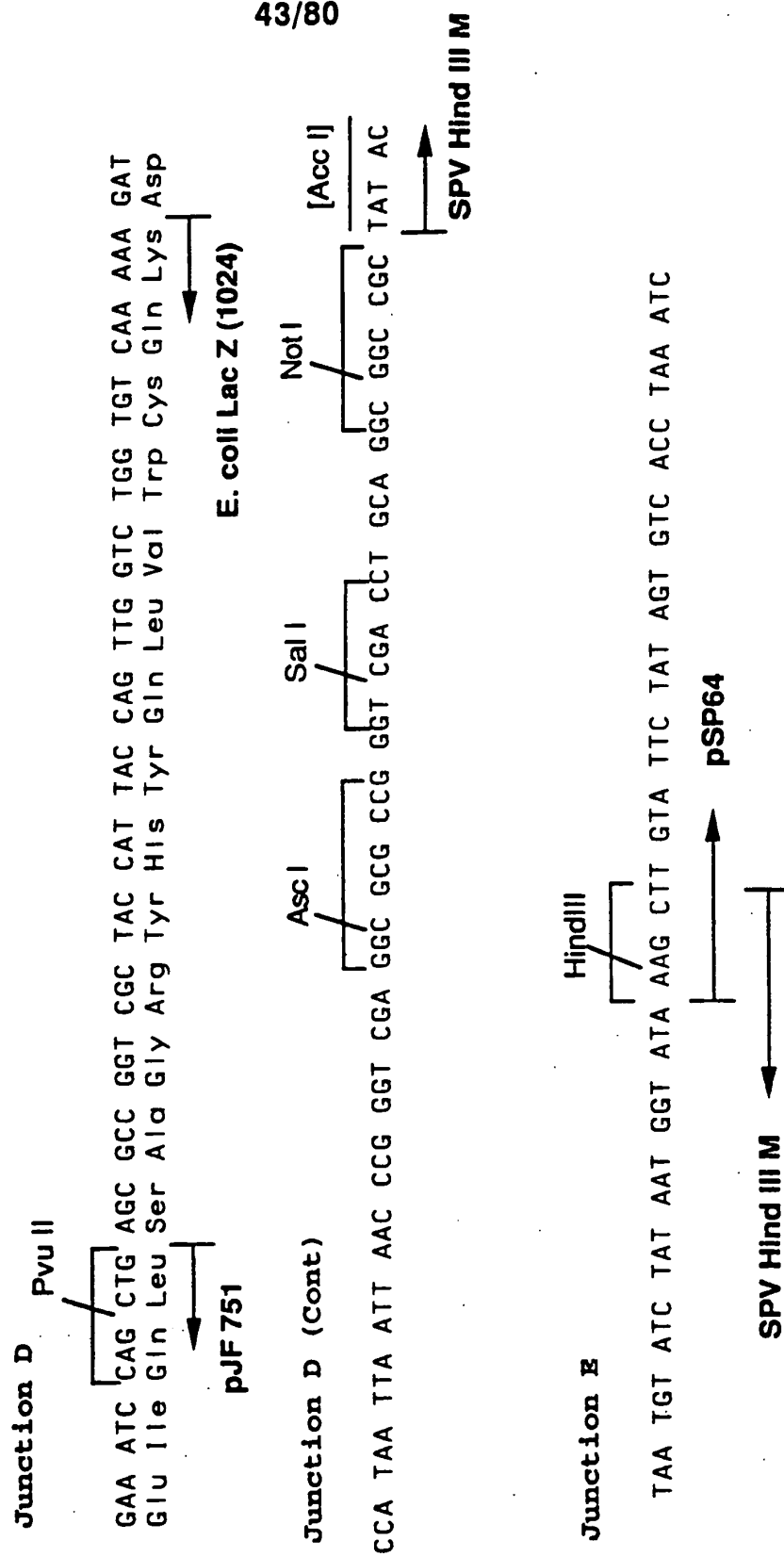
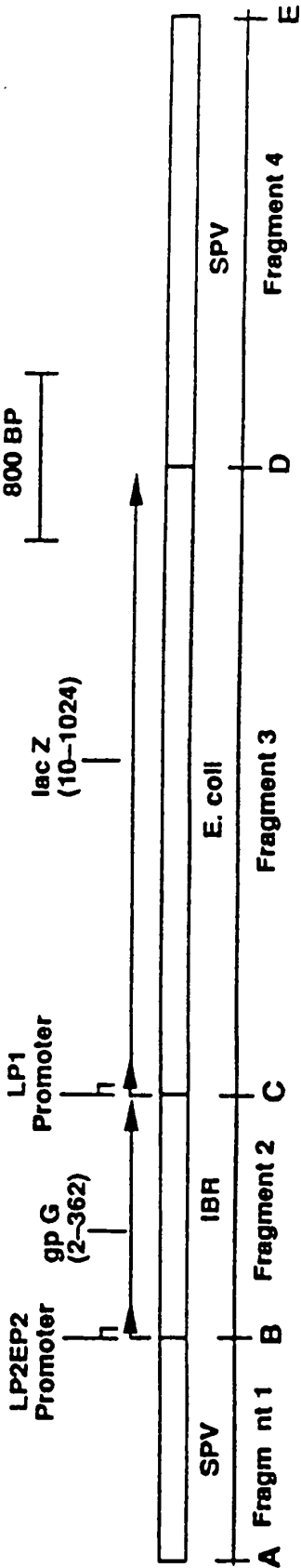


FIGURE 15A  
FIGURE 15B  
FIGURE 15C  
FIGURE 15D

FIGURE 15A

| DNA        | Origin           | Sites           | Size     |
|------------|------------------|-----------------|----------|
| Vector     | pSP64            | Hind III—Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M    | Bgl II—Acc I    | ~1484 BP |
| Fragment 2 | IBR-000 (Cooper) | Eco RI†—Bam HI† | ~1085 BP |
| Fragment 3 | pJF751           | Bam HI—Pvu II   | ~3002 BP |
| Fragment 4 | SPV HindIII M    | Acc I—Hind III  | ~2149 BP |

†Restriction sites introduced by PCR cloning





## Junction A

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**Not I**

[Acc I]

Sal I Xba I

**Xba I**

**LP2**

GTC GAC TCT AGA TTT TTT TTT TTT TTG GCA TAT AAA

SPV Hind III M

### Junction B (Cont)

## Bgl II

**EP2**

**EOPI**

EP2

TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA ATG AAT TCC  
MET Asn Ser CCT GCC GCC CGG  
Pro Ala Ala Arg

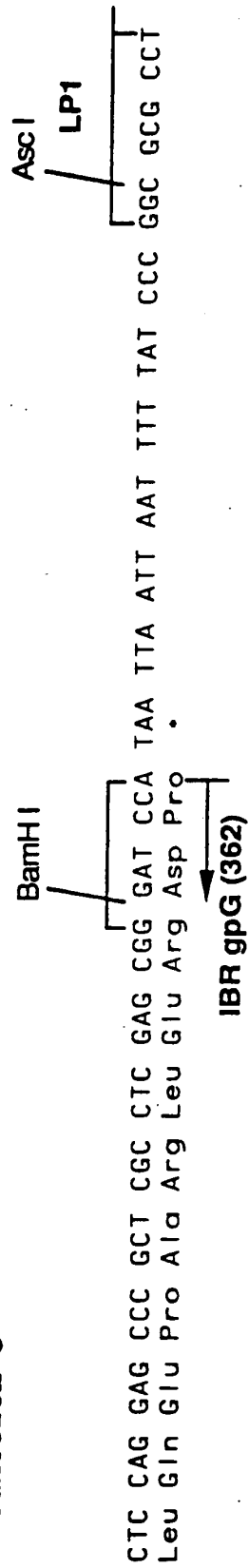
IBR gpG (2)

**IBR gpG (2)**

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FIGURE 15C

Junction C



Junction C (Cont)

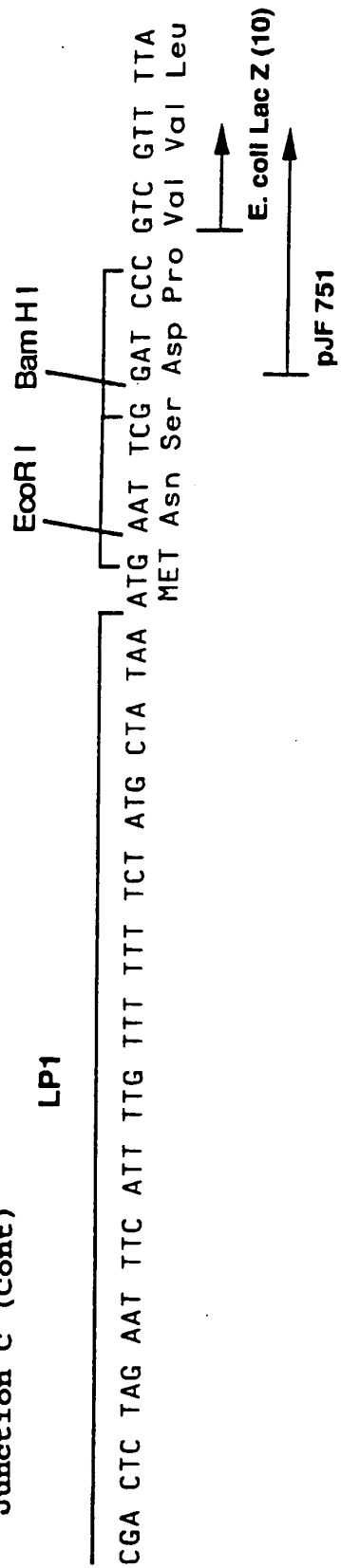
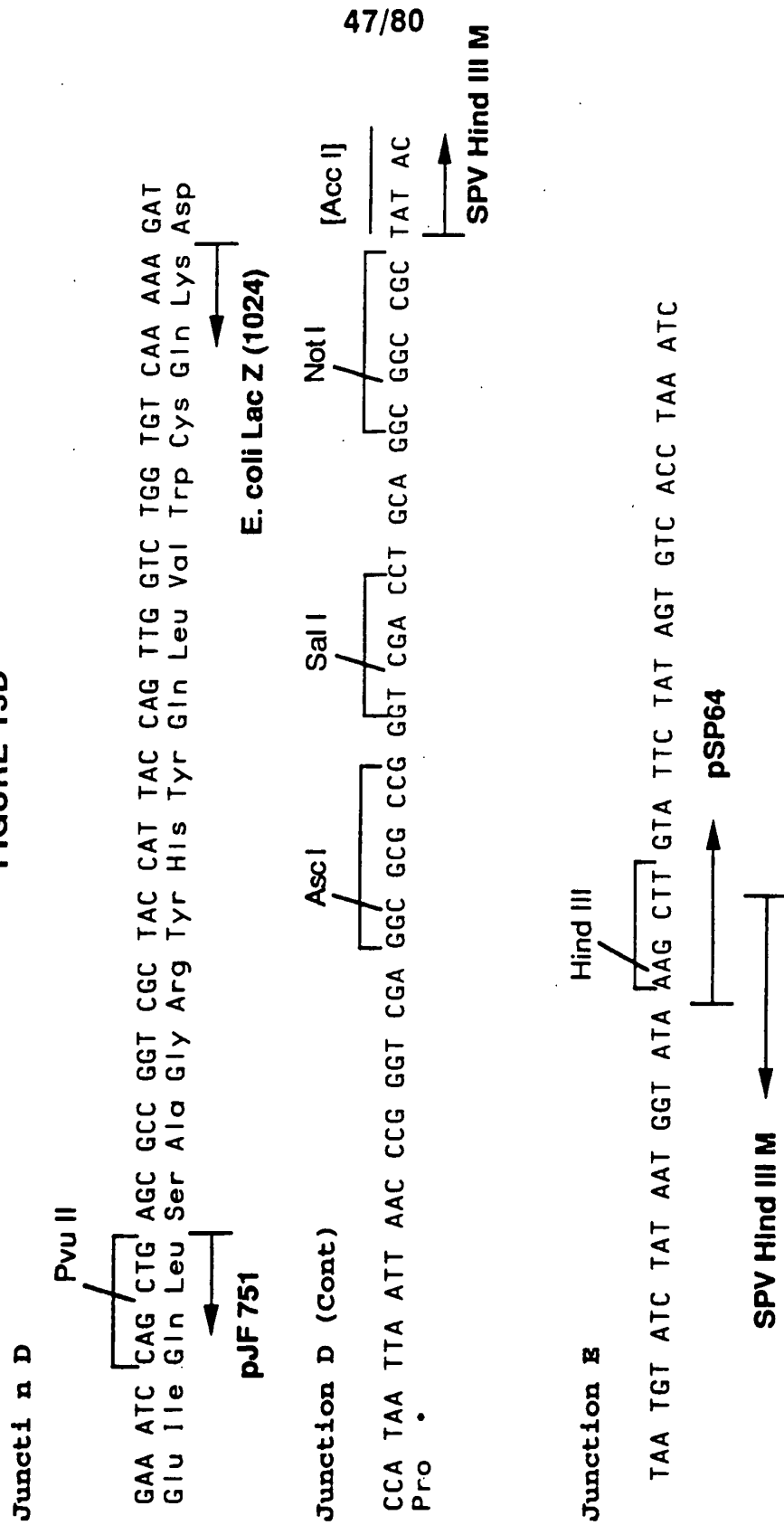


FIGURE 15D



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FIGURE 16

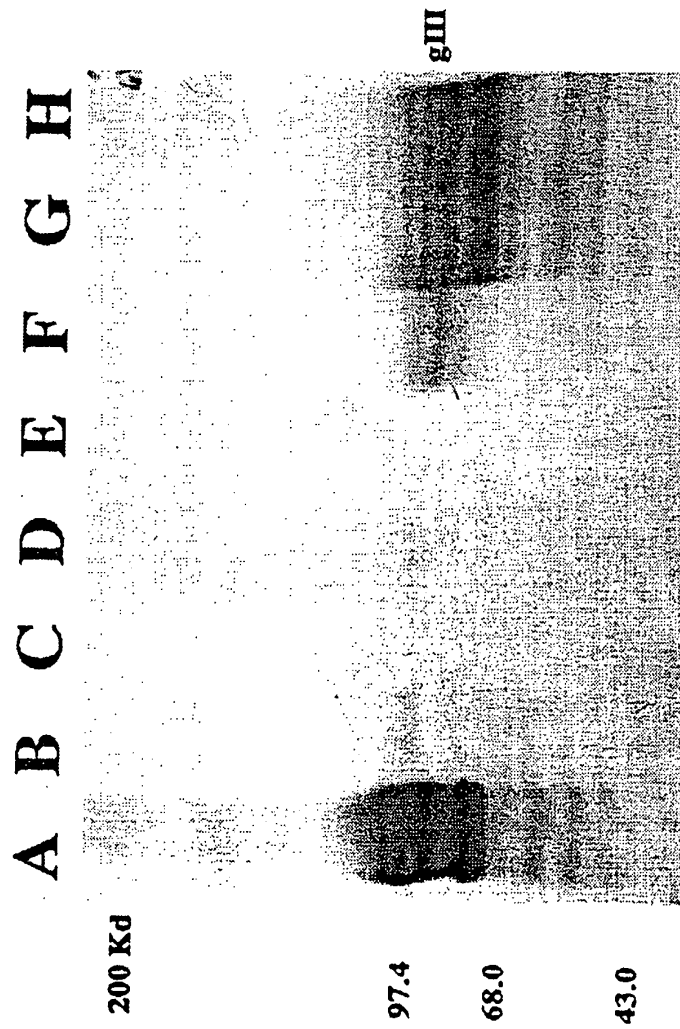


FIGURE 17

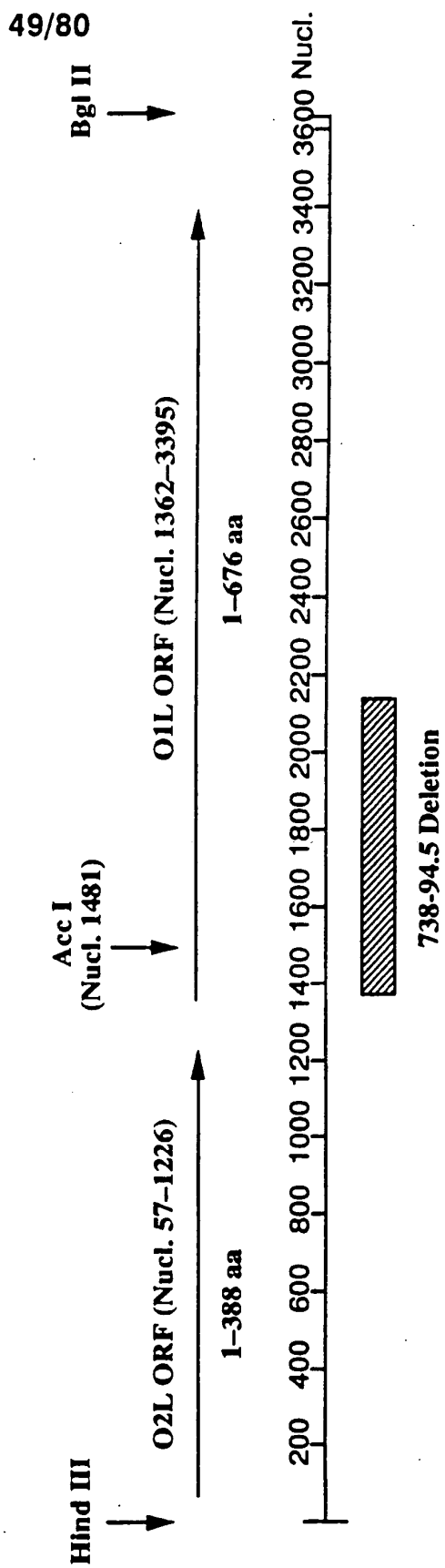
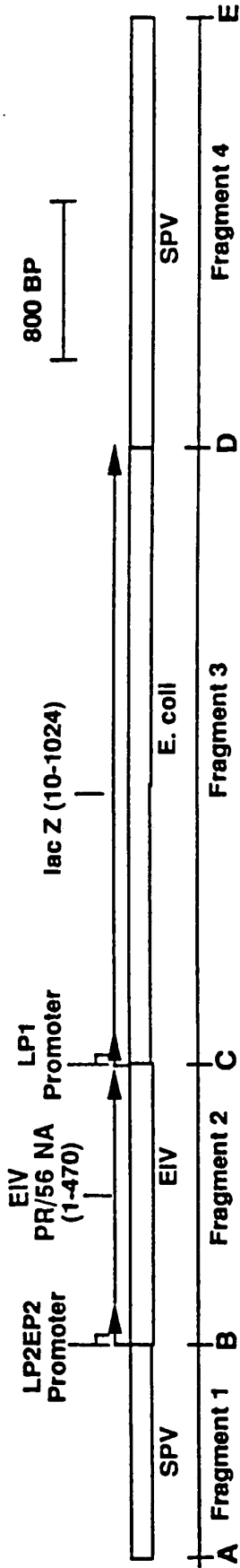


FIGURE 18A  
FIGURE 18B  
FIGURE 18C  
FIGURE 18D

FIGURE 18A

| DNA        | Origin         | Sites           | Size     |
|------------|----------------|-----------------|----------|
| Vector     | pSP64          | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV Hind III M | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | EIVA PR/56 NA  | BamH I†-BamH I† | ~1450 BP |
| Fragment 3 | pJF751         | Bam HI-Pvu II   | ~3010 BP |
| Fragment 4 | SPV Hind III M | Acc I-Hind III  | ~2149 BP |

†Restriction sites introduced by PCR cloning



## Junction A

[Bam H1/Bgl II]

ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

**SPV Hind III M**

**pSP64**

**Not.**

Not I

[Acc I]

**GTAT AGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTG GCA TAT AAA**

Sail Xball

**Xba I**

**LP2**

**Bgl II**

**Junction B  
Continued**

**2d3**

TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA AT

**[EcoR I, BamH I]**

**Junction B  
Continued**

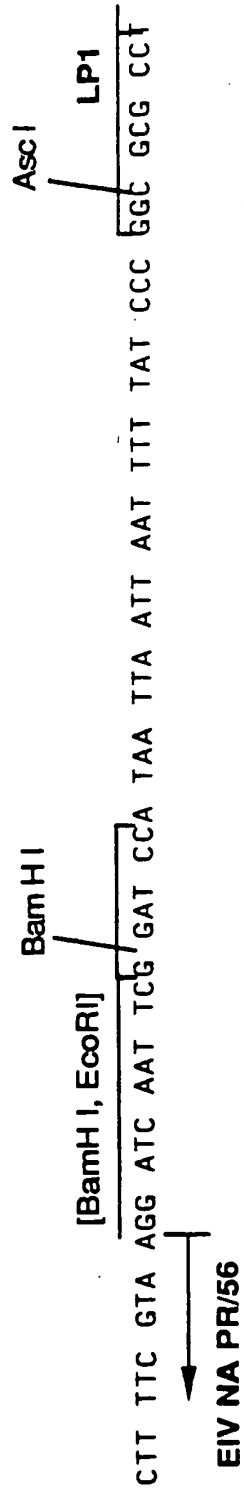
GAAT TTG ATC CAT GAA TCC TAAT

**EIV NA PR/56 (55-452)**

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FIGURE 18C

Junction C



Junction C Continued

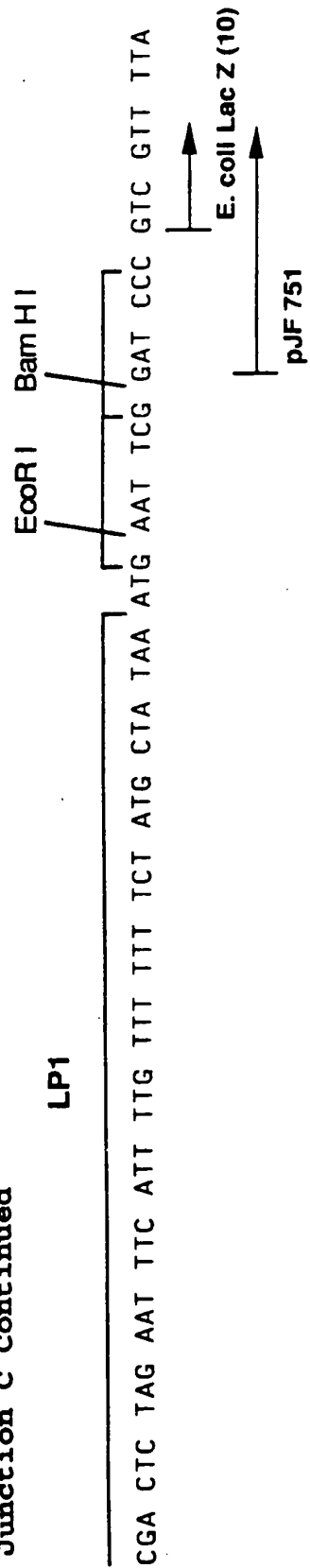




FIGURE 18D

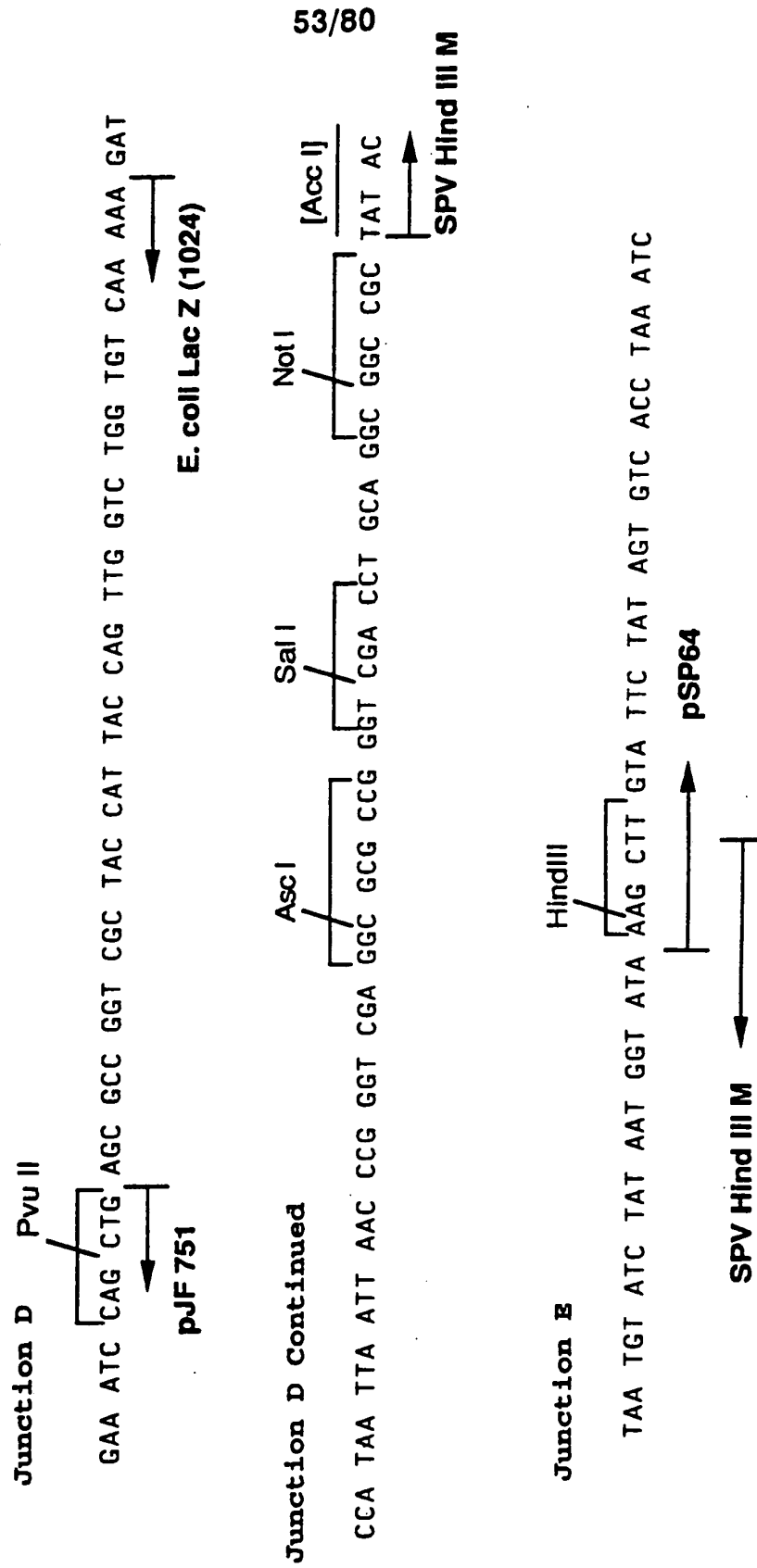
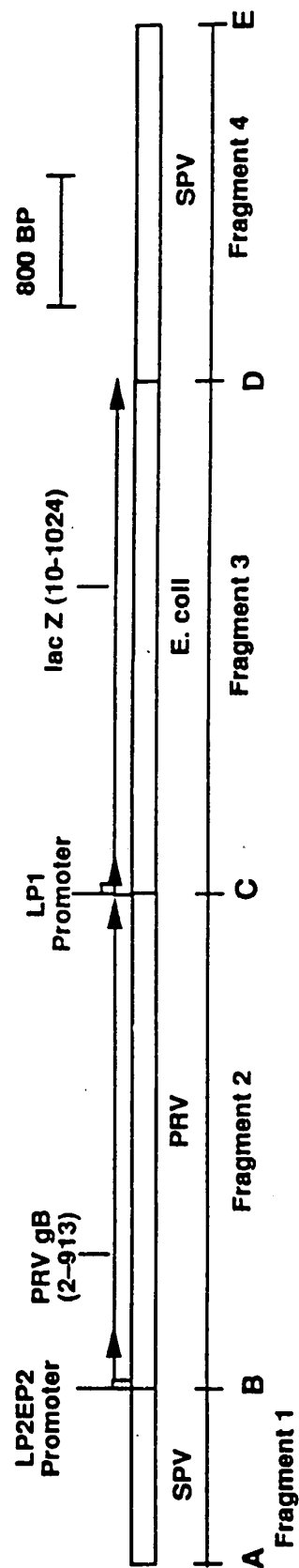


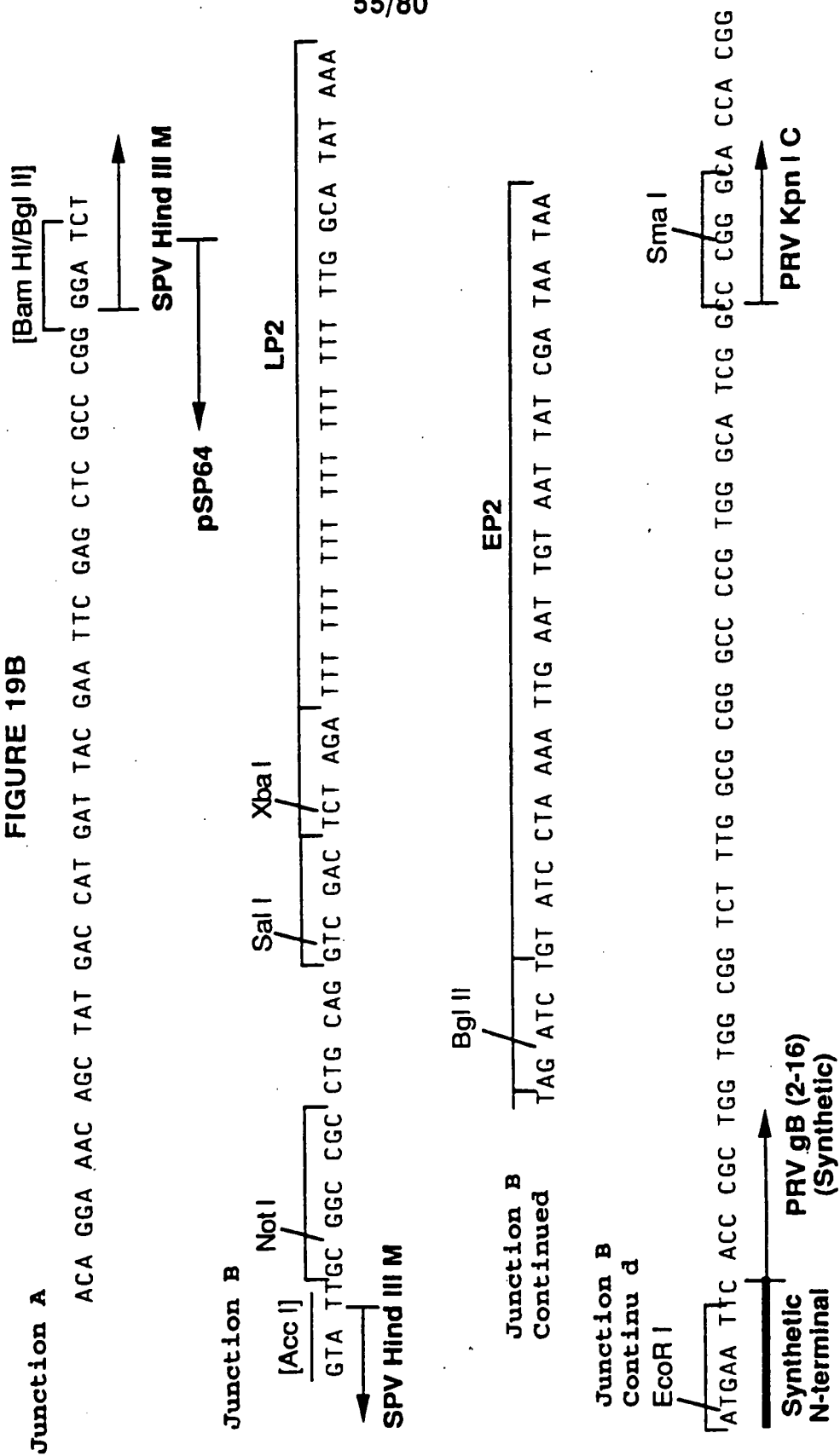
FIGURE 19A  
FIGURE 19B  
FIGURE 19C  
FIGURE 19D

FIGURE 19A

| DNA        | Origin         | Sites           | Size     |
|------------|----------------|-----------------|----------|
| Vector     | pSP64          | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV Hind III M | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | PRV Kpn I C    | Sma I-Sac I     | ~3500 BP |
| Fragment 3 | pJF751         | Bam HI-Pvu II   | ~3010 BP |
| Fragment 4 | SPV Hind III M | Acc I-Hind III  | ~2149 BP |

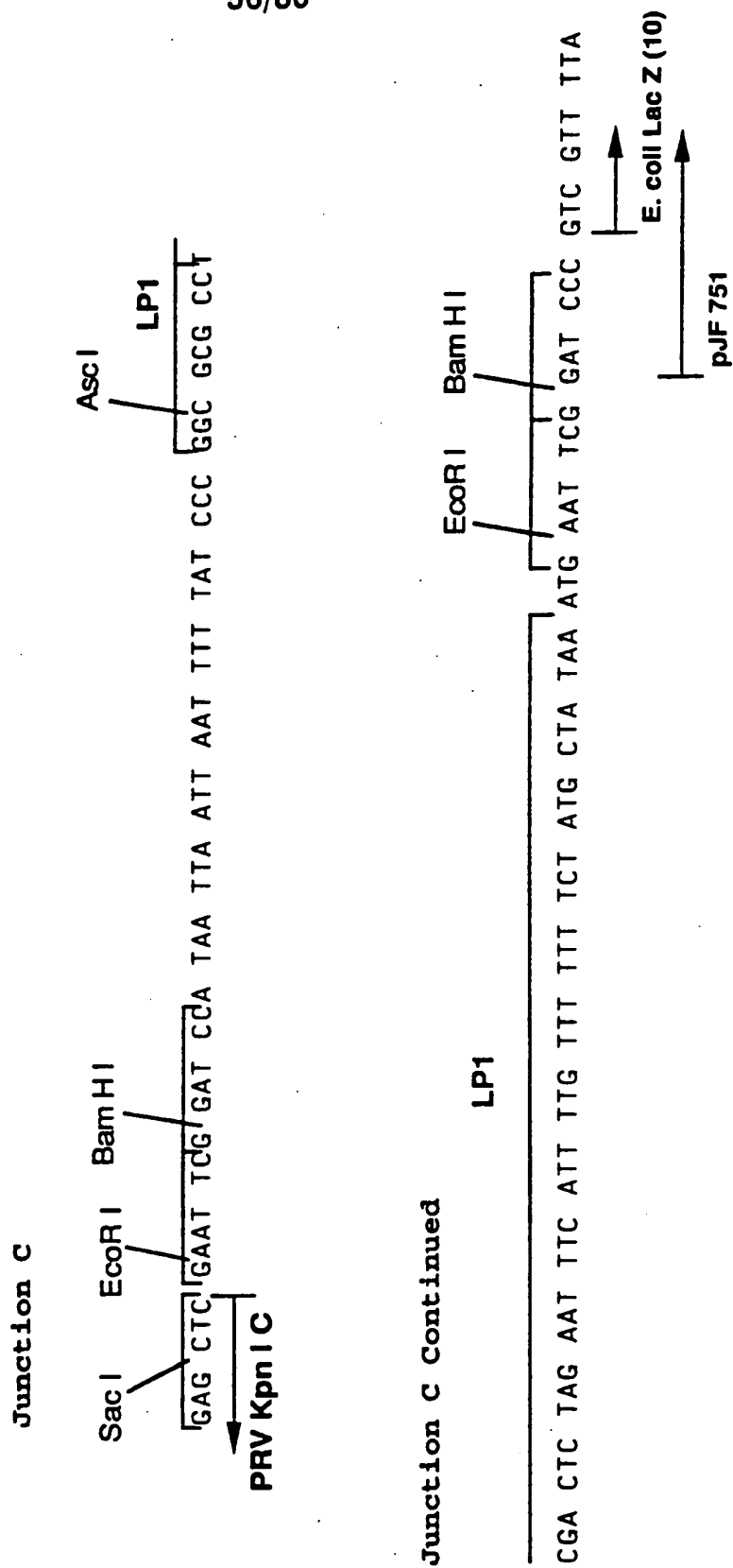


**[Bam H1/Bgl II]**



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FIGURE 19C



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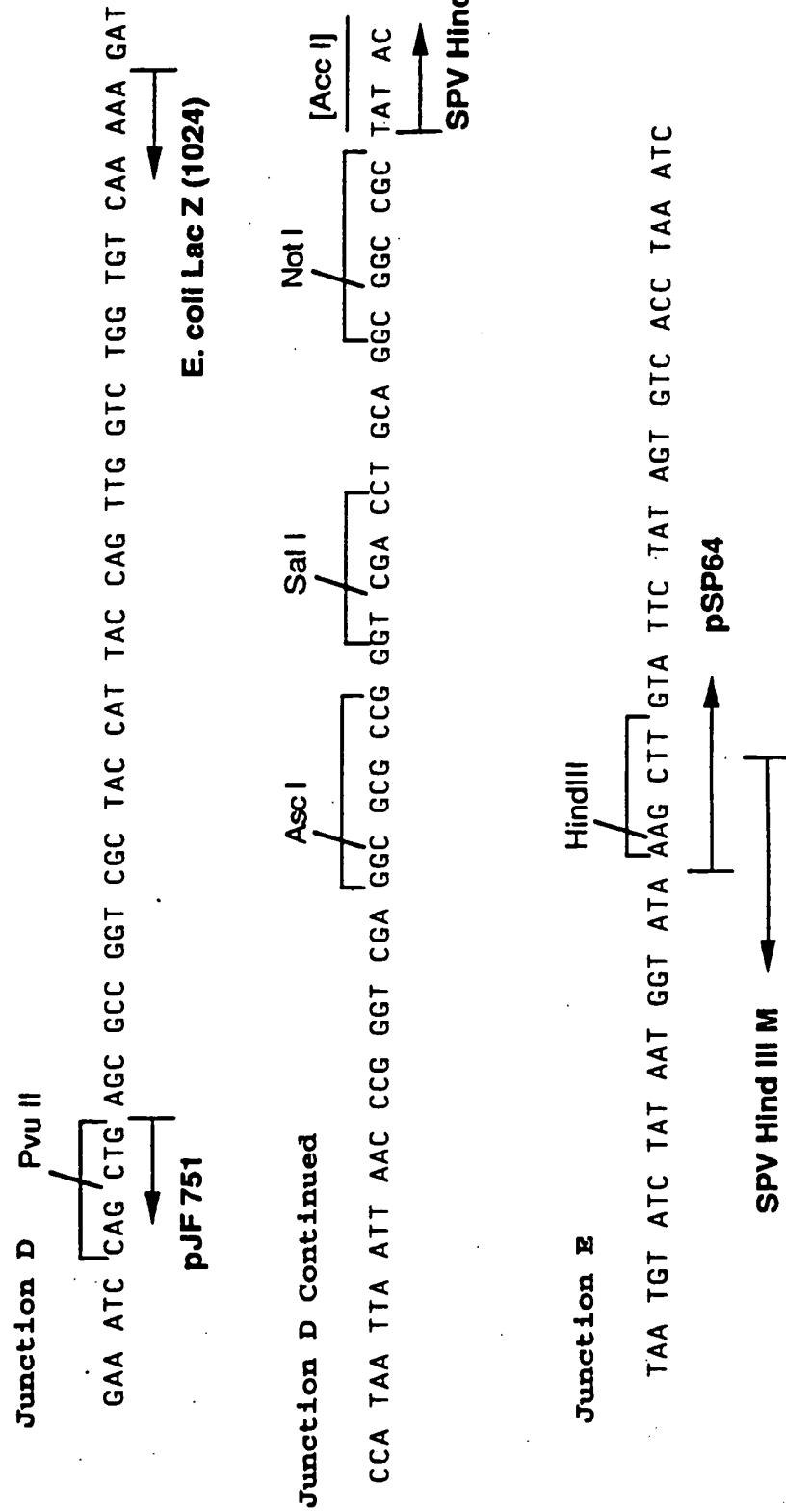


FIGURE 20A  
FIGURE 20B  
FIGURE 20C  
FIGURE 20D

FIGURE 20A

| DNA        | Origin        | Sites           | Size     |
|------------|---------------|-----------------|----------|
| Vector     | pSP64         | Hind III—Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M | Bgl II—Acc I    | ~1484 BP |
| Fragment 2 | pJF751        | Bam HI—Pvu II   | ~3002 BP |
| Fragment 3 | Hepatitis B   | BamH I—EcoR I   | ~ 589 BP |
| Fragment 4 | SPV HindIII M | Acc I—Hind III  | ~2149 BP |

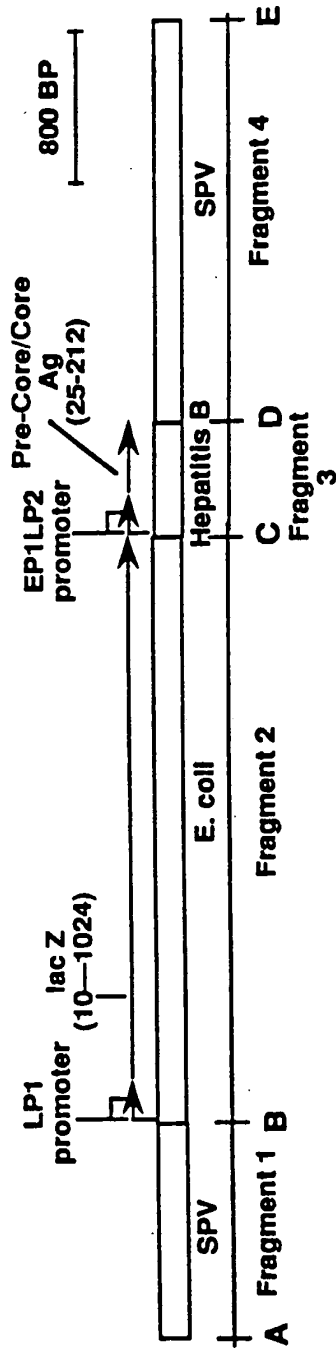


FIGURE 20B

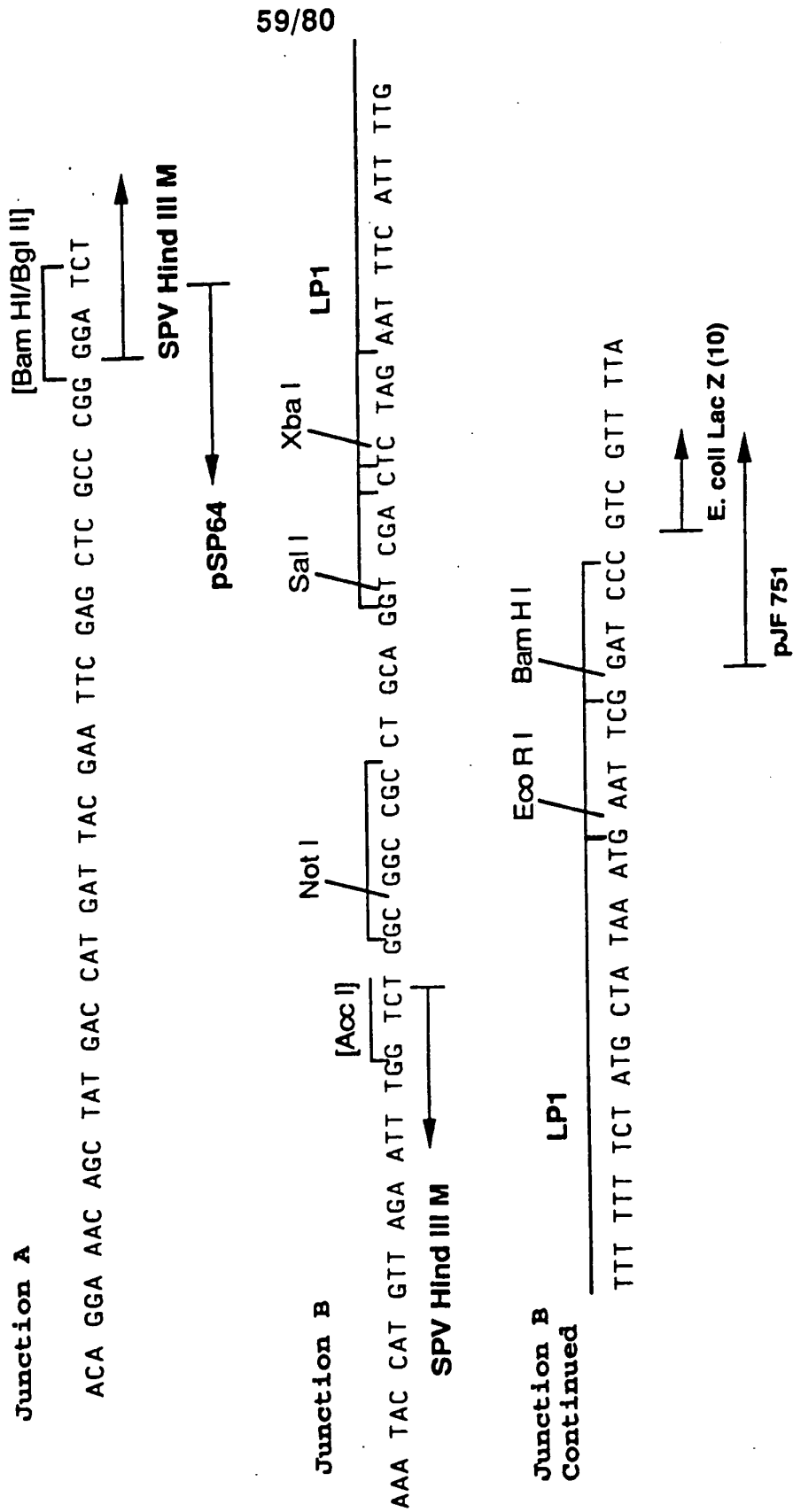
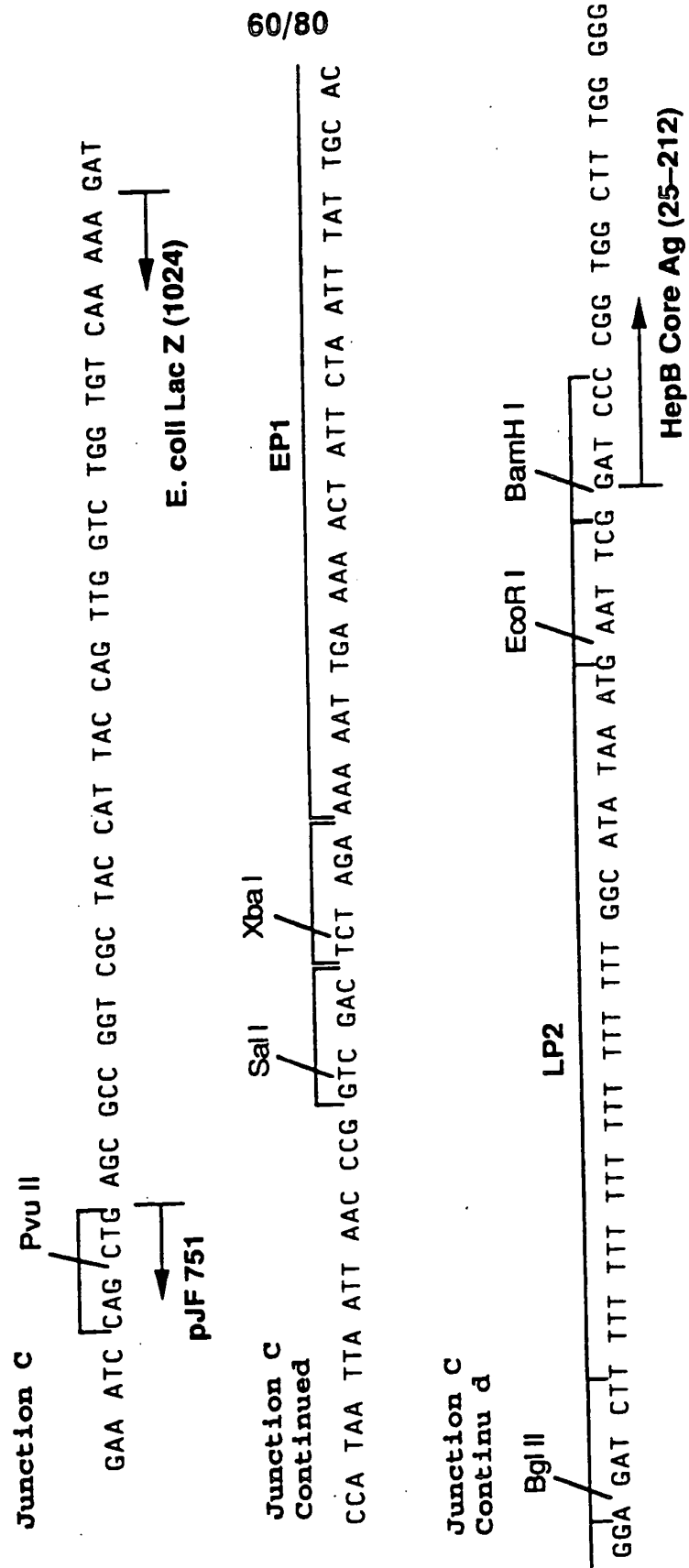


FIGURE 20C





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FIGURE 20D

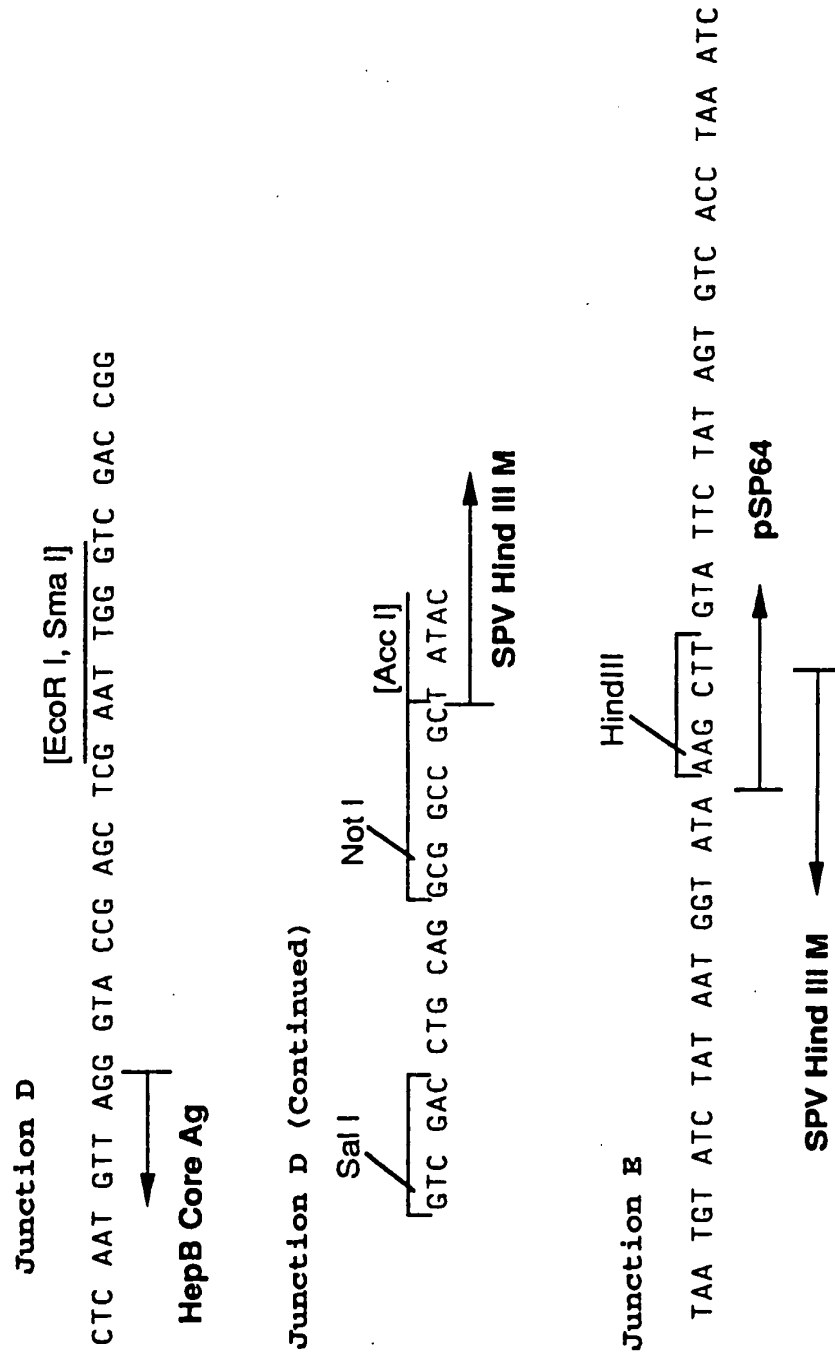
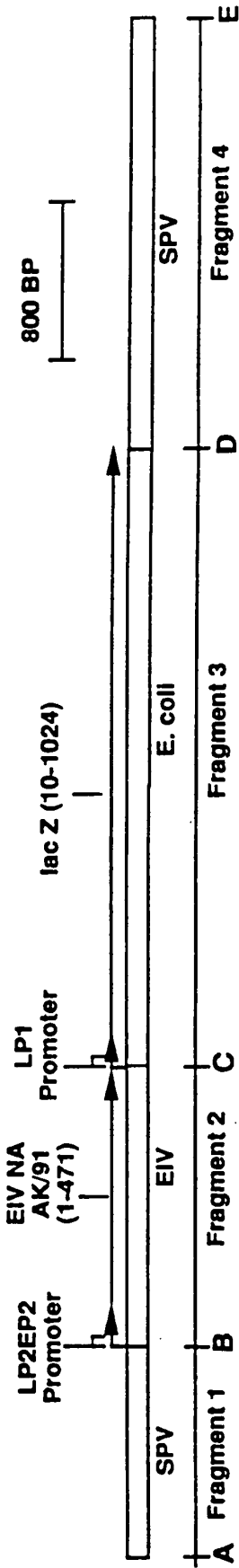


FIGURE 21A  
FIGURE 21B  
FIGURE 21C  
FIGURE 21D

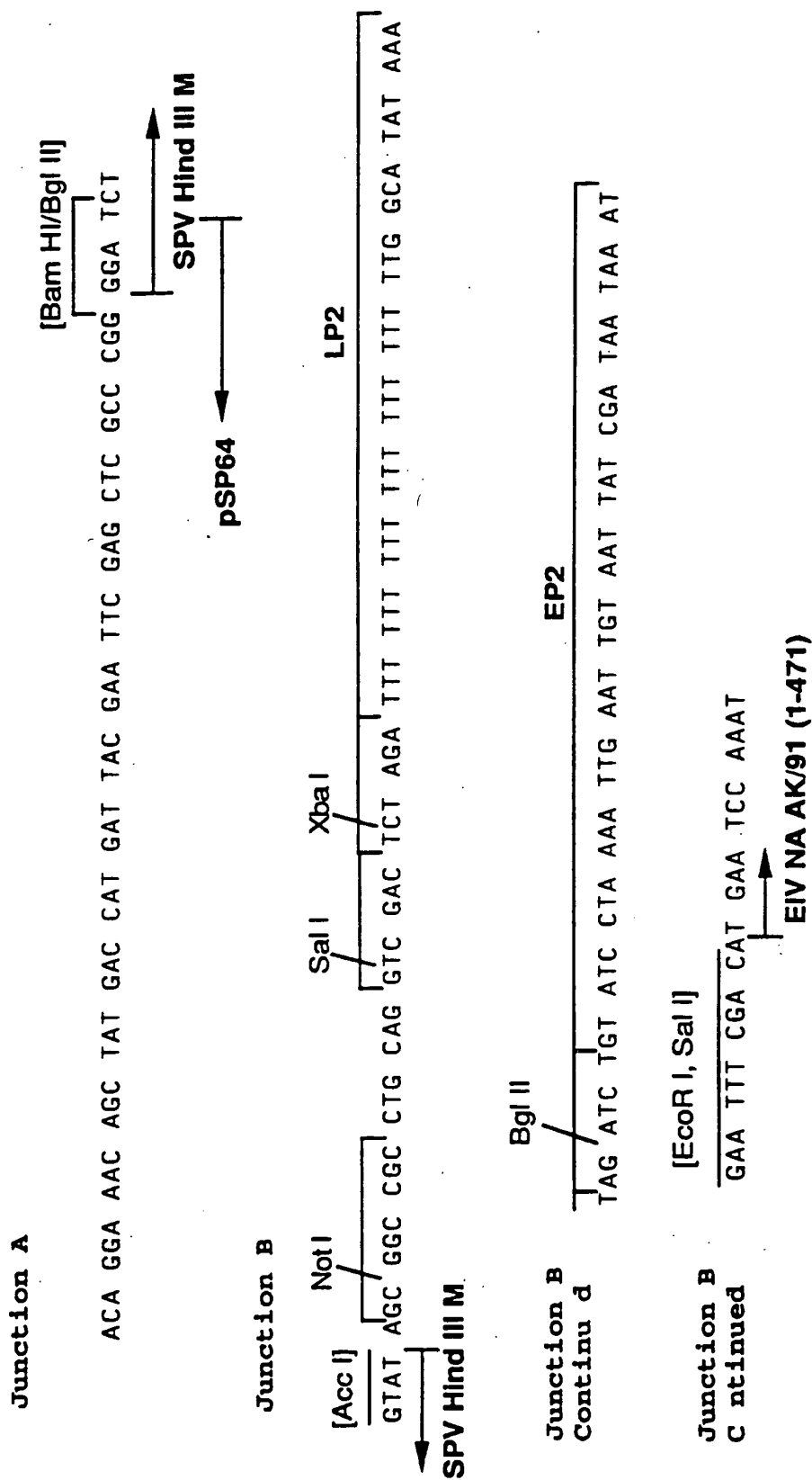
FIGURE 21A

| DNA        | Origin         | Sites           | Size     |
|------------|----------------|-----------------|----------|
| Vector     | pSP64          | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV Hind III M | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | EIVA NA AK/91  | Sal I†-Sal I†   | ~1450 BP |
| Fragment 3 | pJF751         | Bam HI-Pvu II   | ~3010 BP |
| Fragment 4 | SPV Hind III M | Acc I-Hind III  | ~2149 BP |

†Restriction sites introduced by PCR cloning



**FIGURE 21B**



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FIGURE 21C

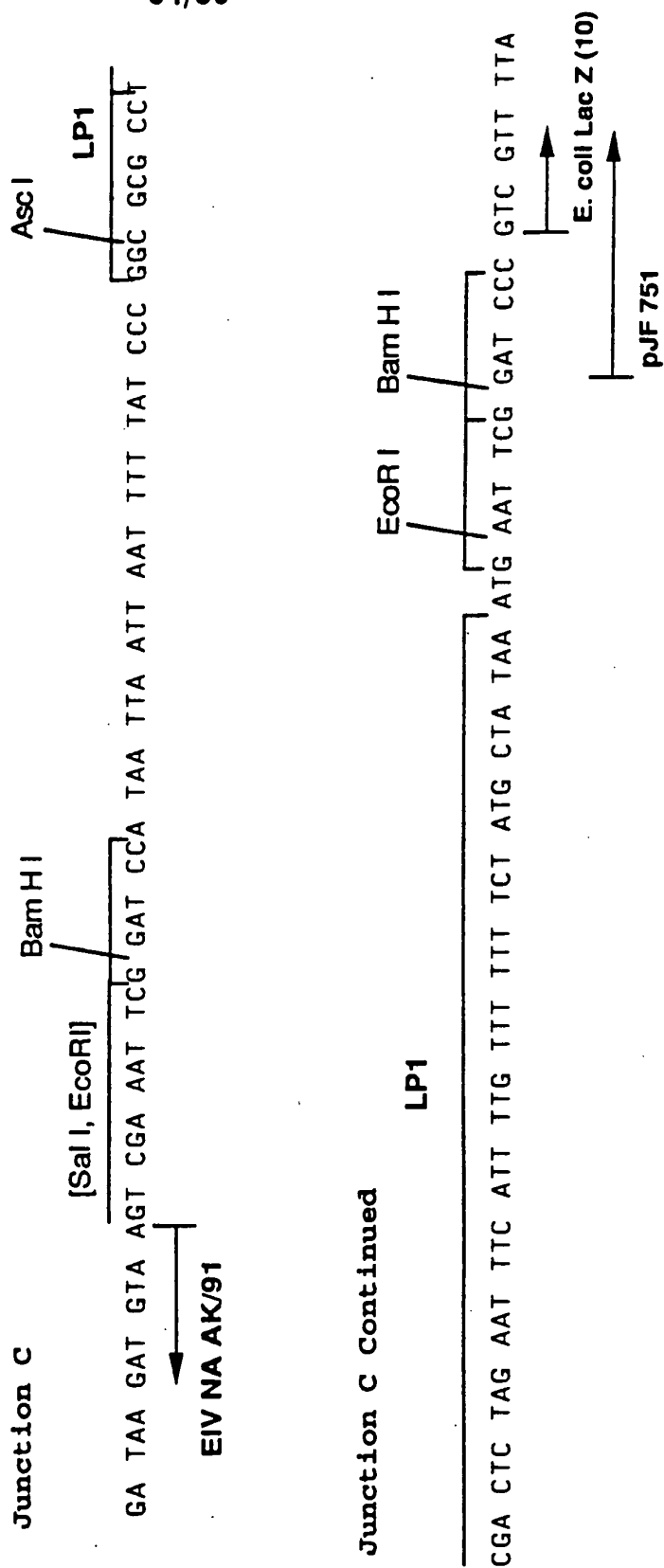
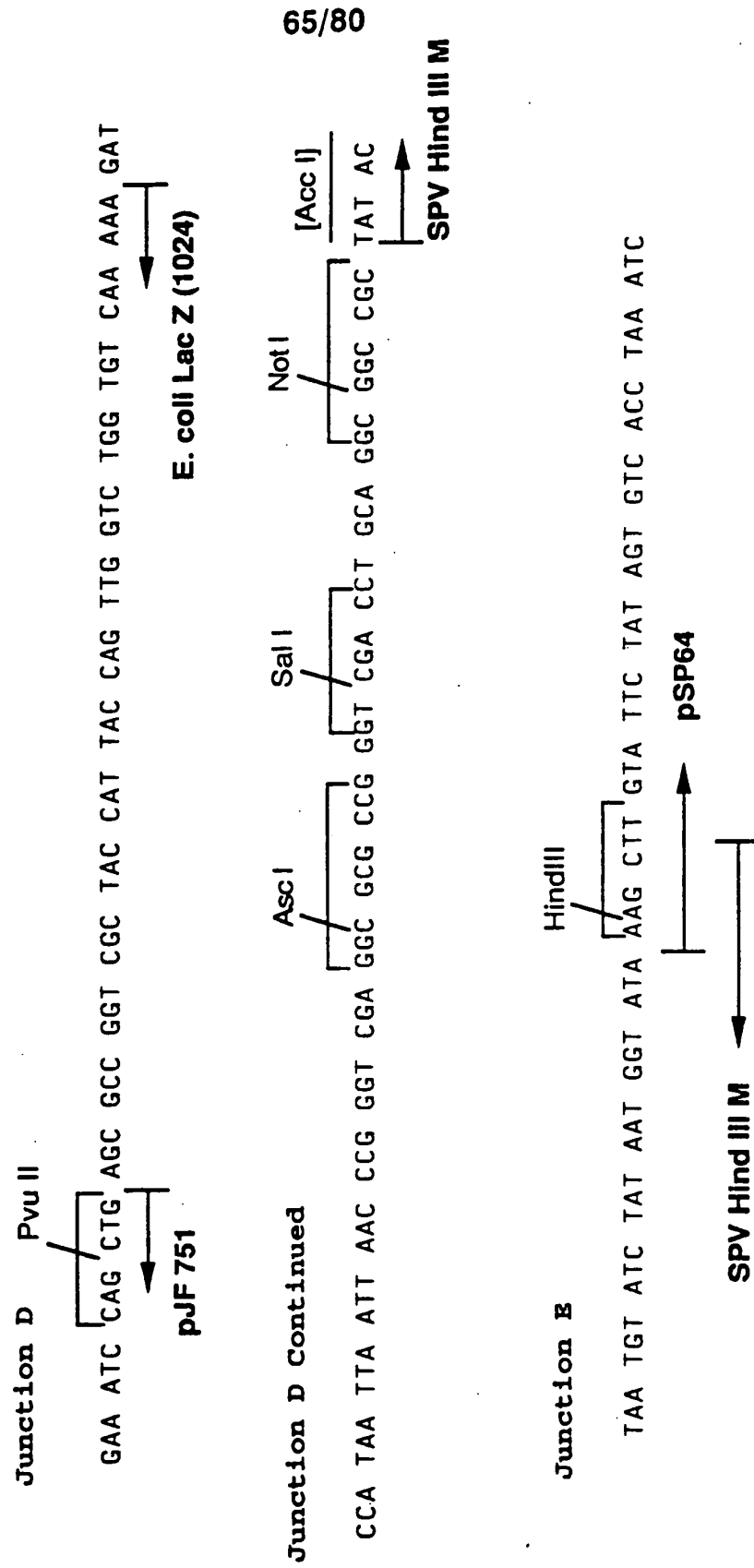


FIGURE 21D



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FIGURE 22A  
FIGURE 22B  
FIGURE 22C

FIGURE 22A

| DNA        | Origin            | Sites           | Size     |
|------------|-------------------|-----------------|----------|
| Vector     | pSP64             | Hind III—Bam HI | ~2972 BP |
| Fragment 1 | SPV HindIII M     | Bgl II—Acc I    | ~1484 BP |
| Fragment 2 | HCMV 2.1 kb Pst I | Pst I—Ava II    | ~1154 BP |
| Fragment 3 | pJF 751           | BamH I—Pvu II   | ~3010 BP |
| Fragment 4 | PRV BamH I #7     | Nde I—Sal I     | ~ 750 BP |
| Fragment 5 | SPV HindIII M     | Acc I—Hind III  | ~2149 BP |

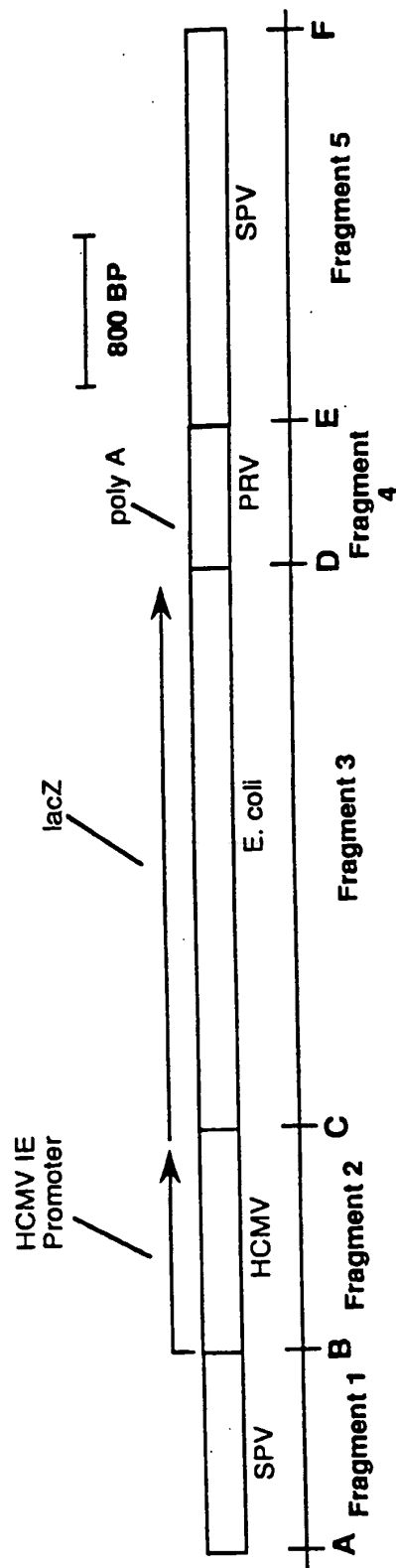
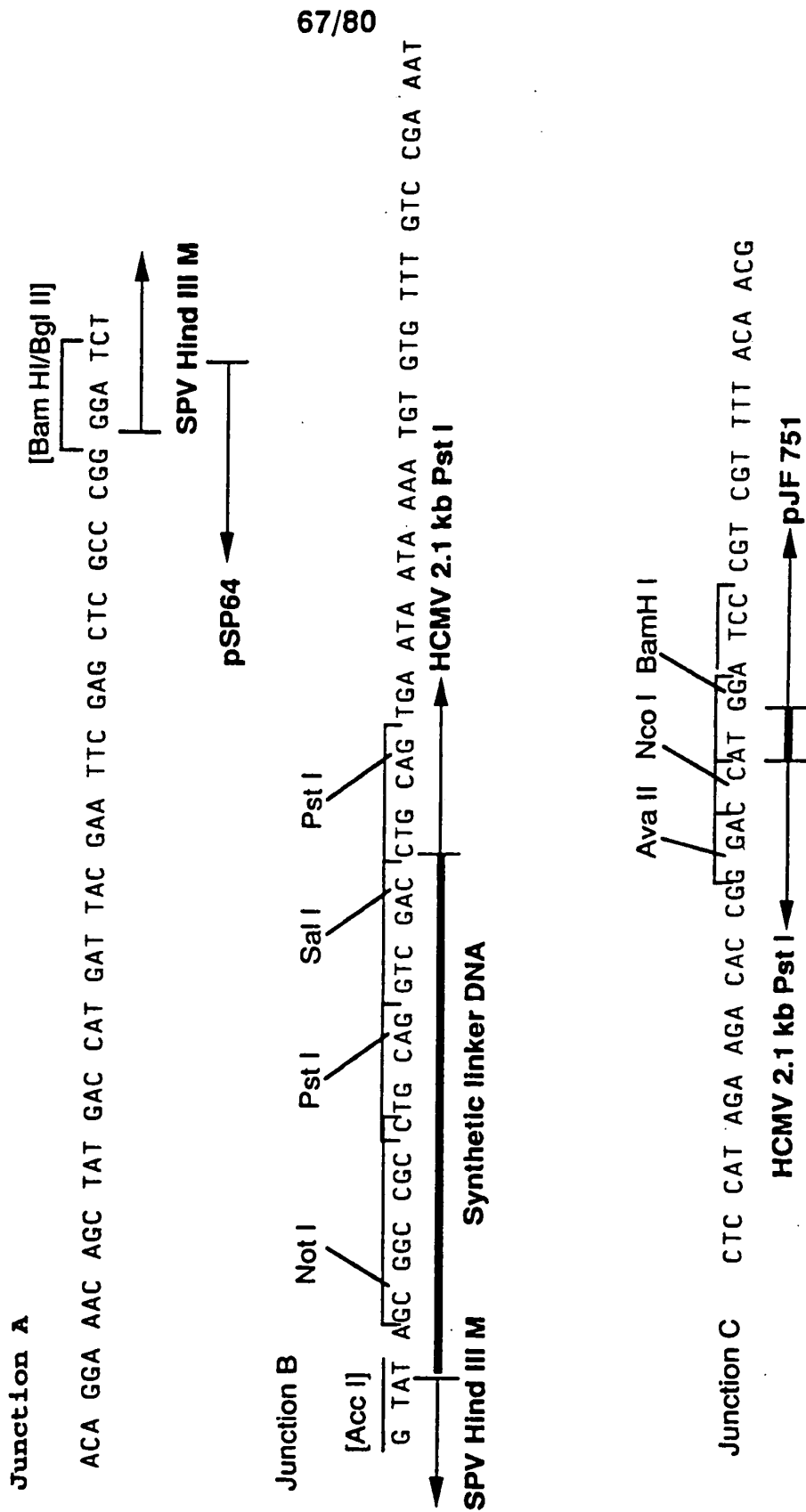


FIGURE 22B



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FIGURE 22C

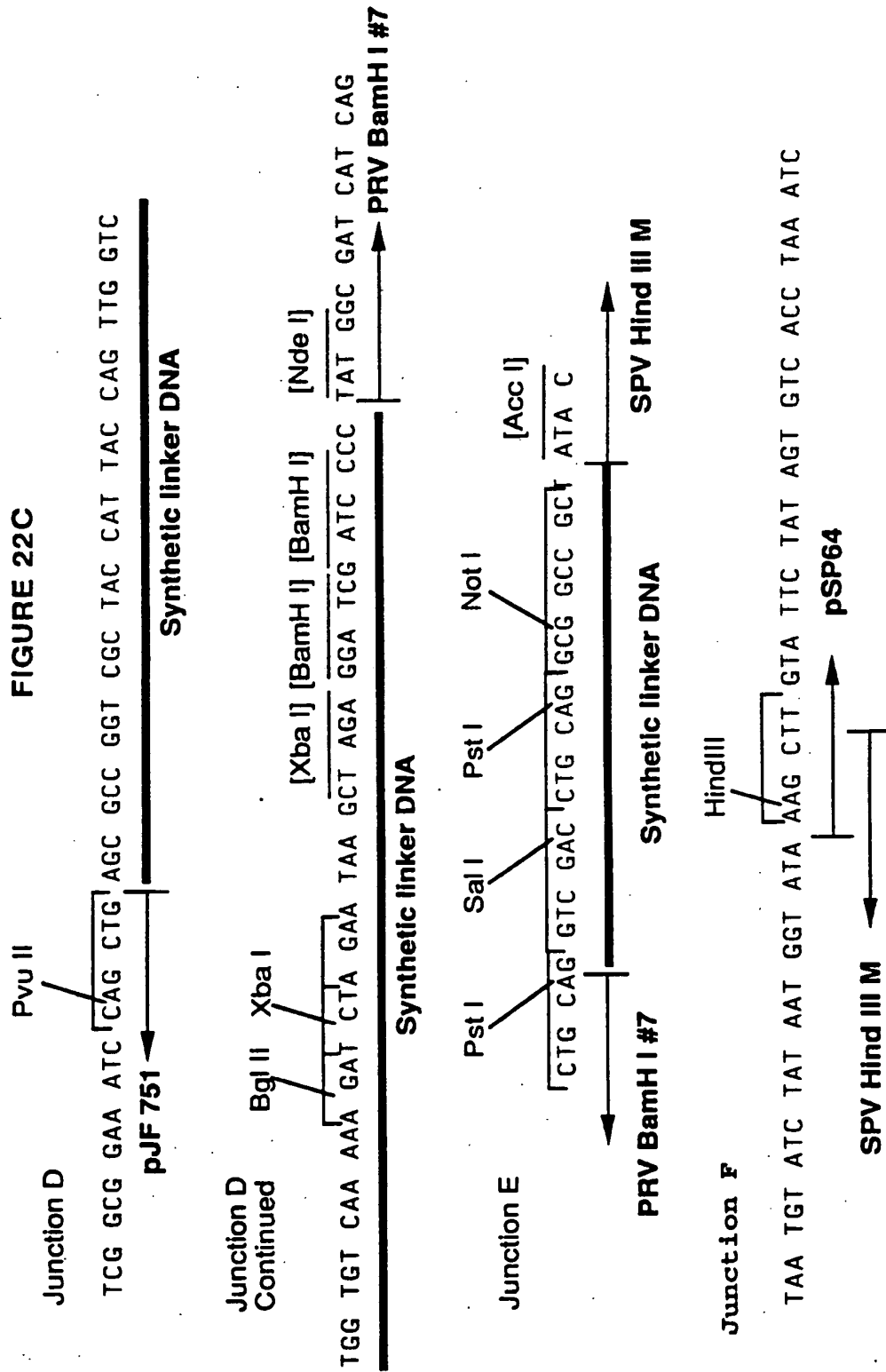


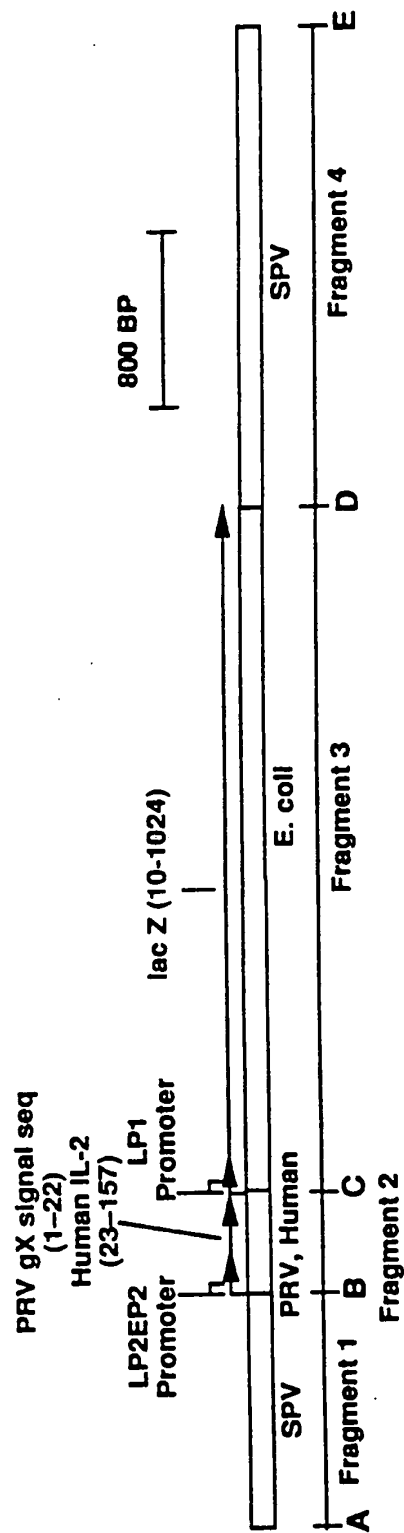


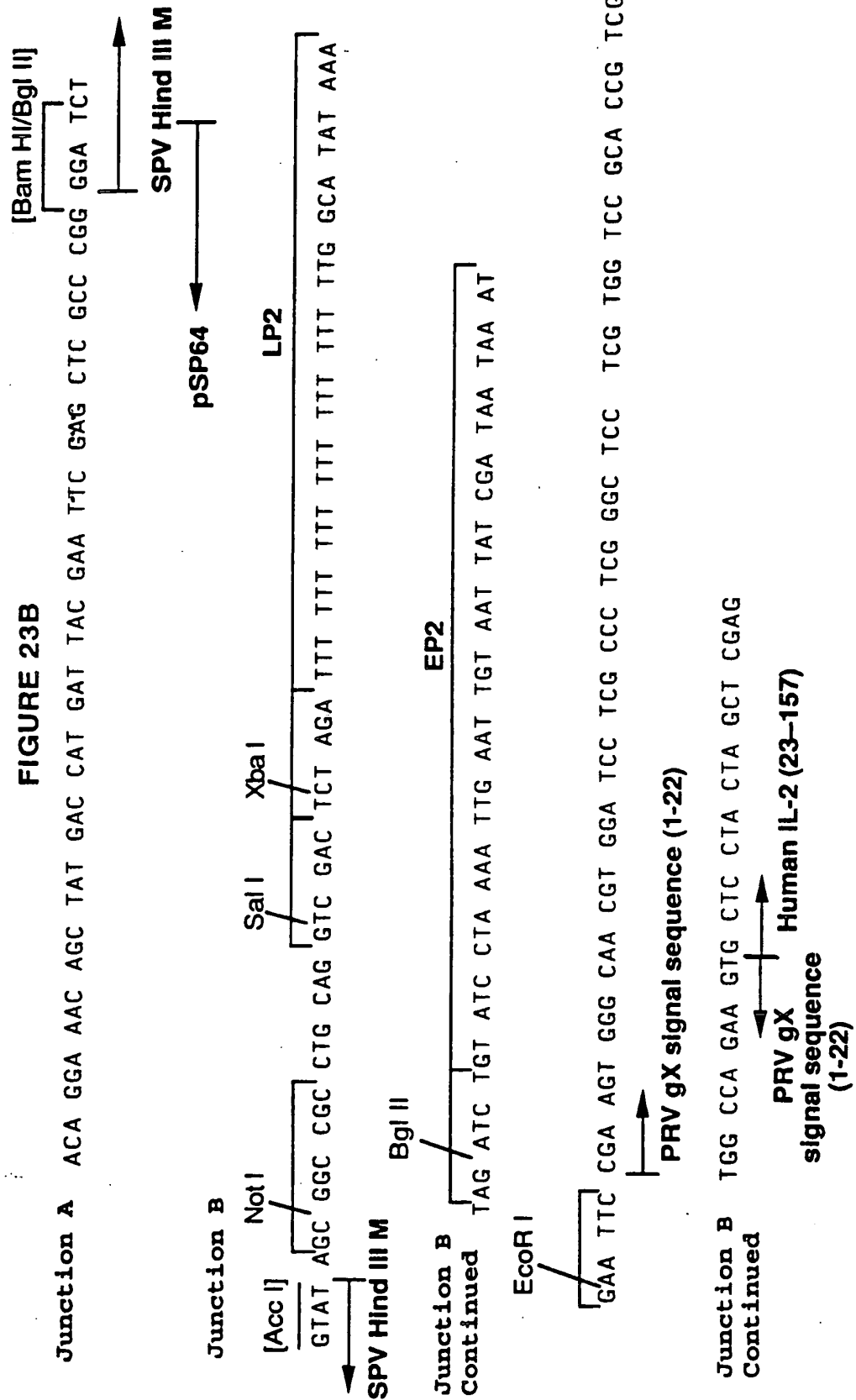
FIGURE 23A  
FIGURE 23B  
FIGURE 23C  
FIGURE 23D

FIGURE 23A

| DNA        | Origin         | Sites           | Size     |
|------------|----------------|-----------------|----------|
| Vector     | pSP64          | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV Hind III M | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | PRV, Human     | EcoR I†-Bgl II  | ~475 BP  |
| Fragment 3 | pJF751         | Bam HI-Pvu II   | ~3010 BP |
| Fragment 4 | SPV Hind III M | Acc I-Hind III  | ~2149 BP |

†Restriction sites introduced by PCR cloning

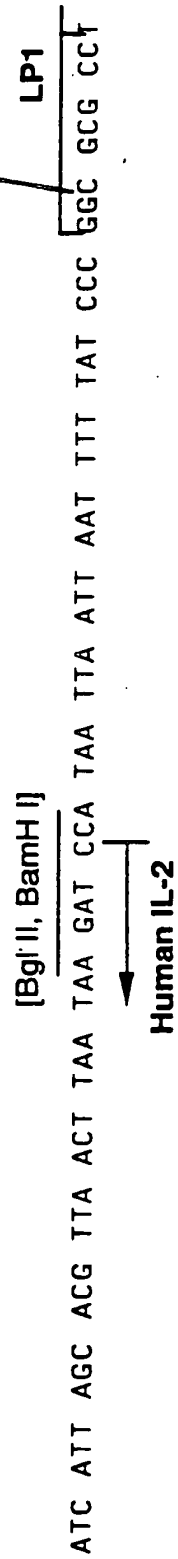




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FIGURE 23C

Junction C



Junction C Continued

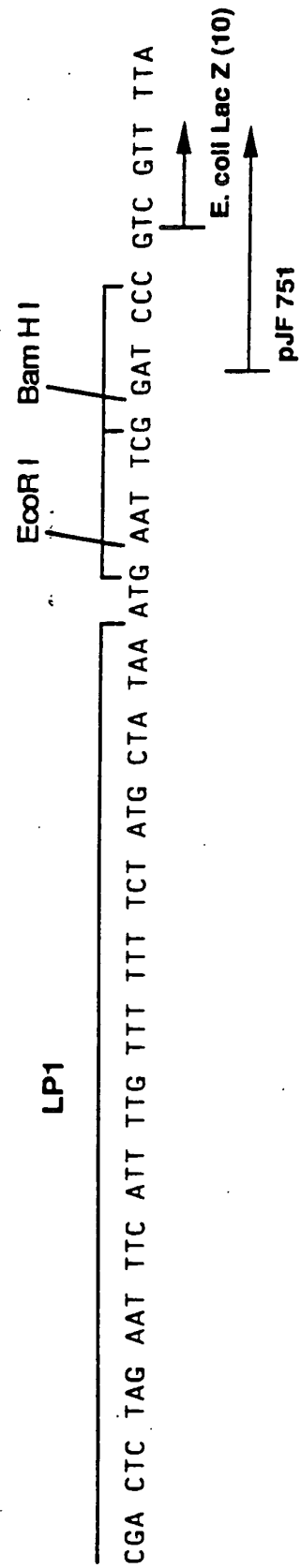
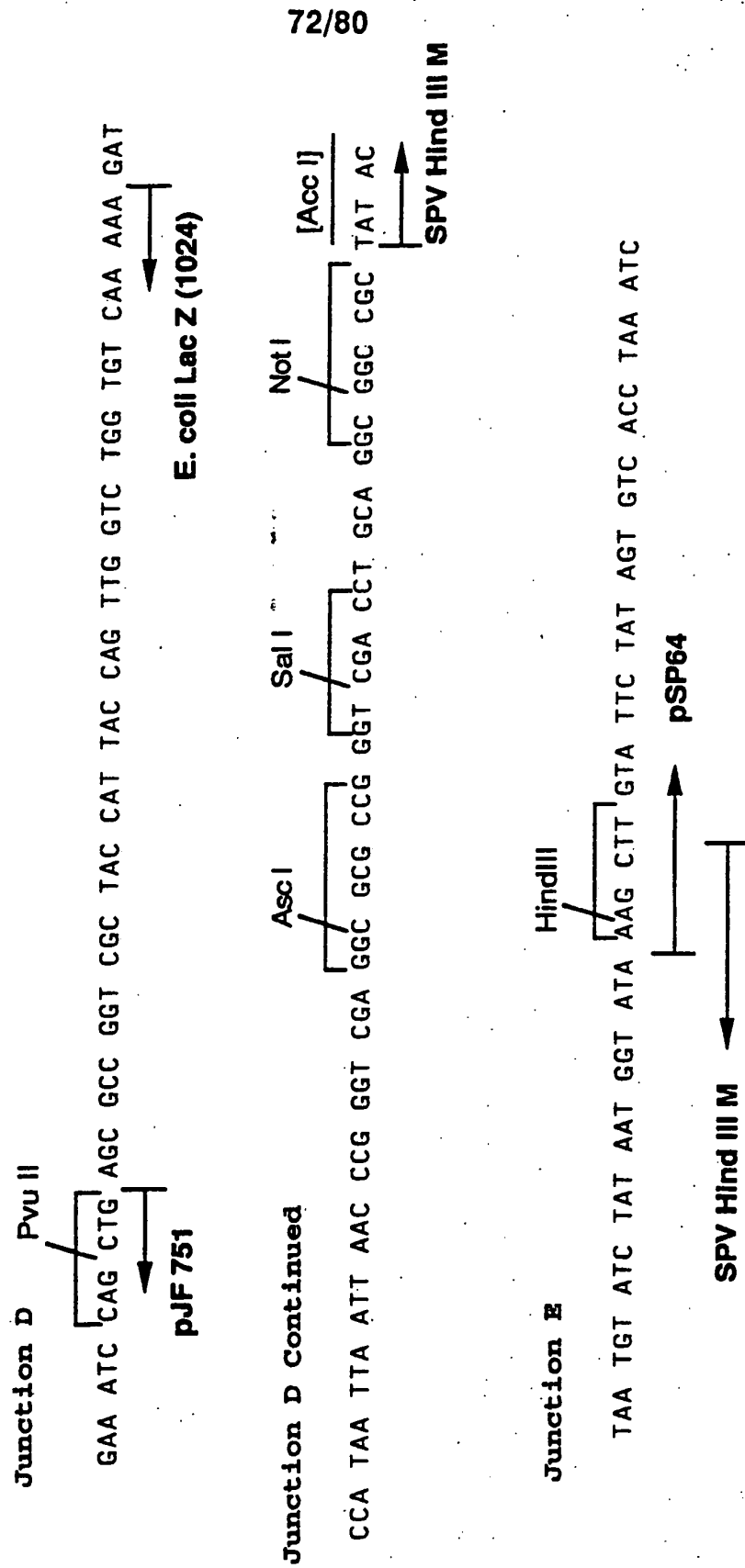


FIGURE 23D



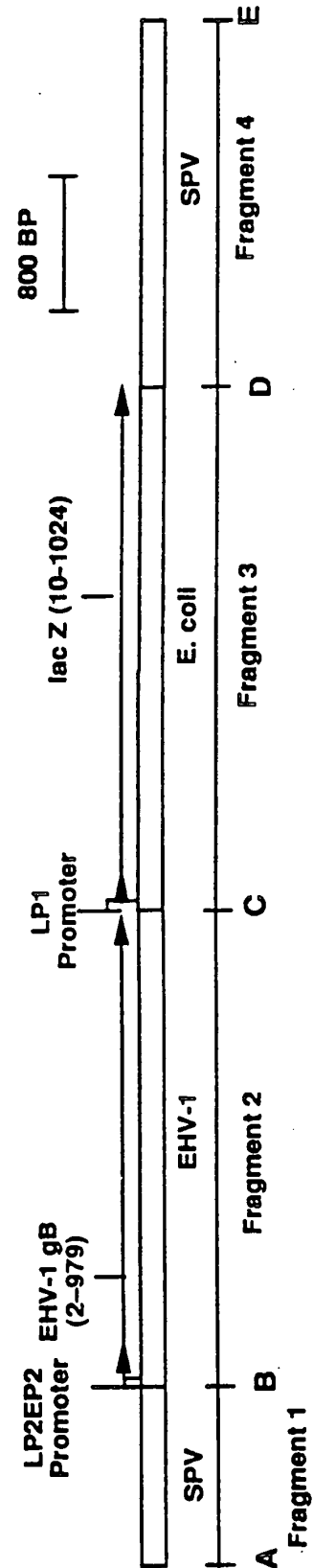
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FIGURE 24A  
FIGURE 24B  
FIGURE 24C  
FIGURE 24D

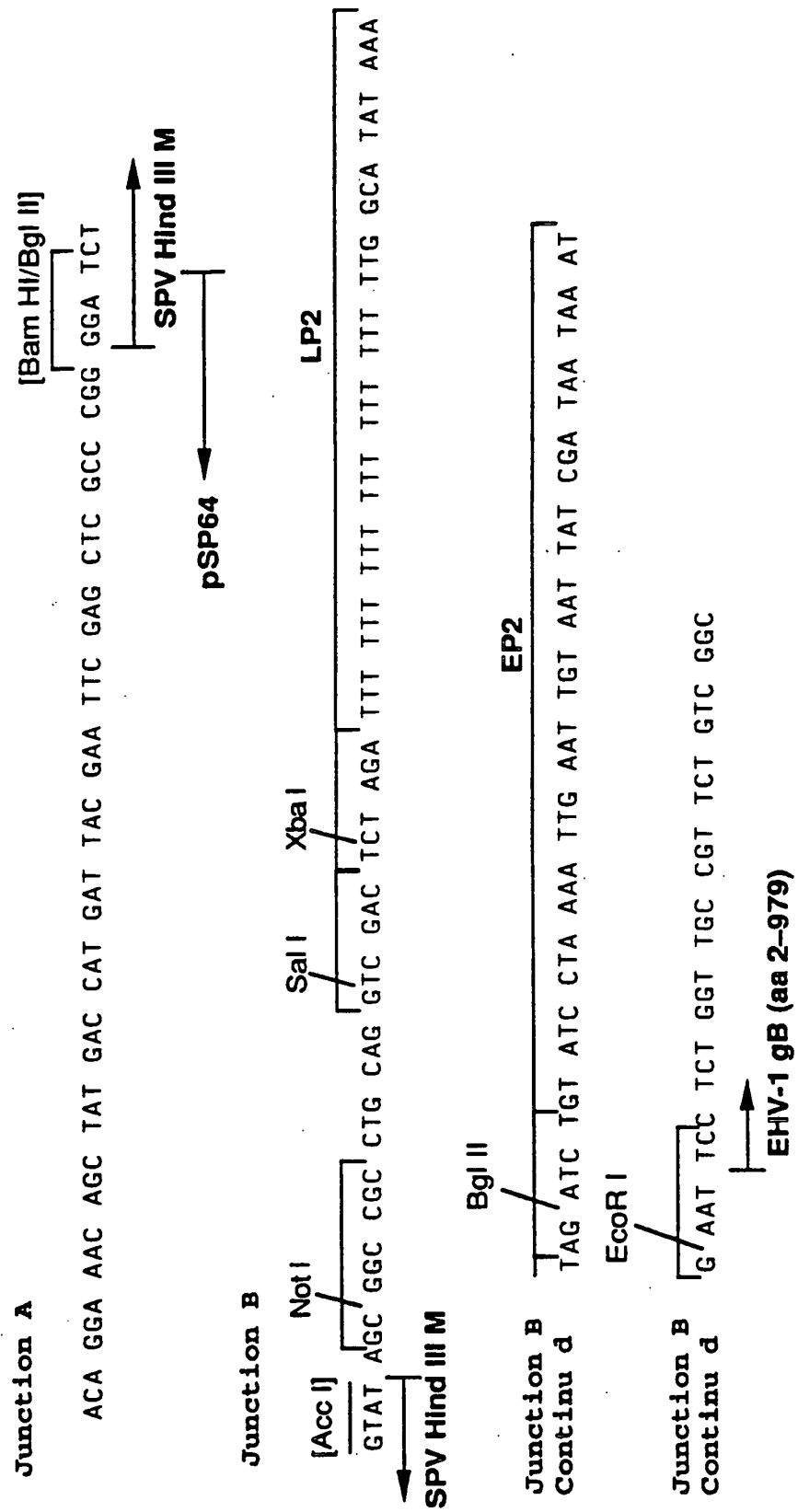
FIGURE 24A

| DNA        | Origin            | Sites           | Size     |
|------------|-------------------|-----------------|----------|
| Vector     | pSP64             | Hind III-Bam HI | ~2972 BP |
| Fragment 1 | SPV Hind III M    | Bgl II-Acc I    | ~1484 BP |
| Fragment 2 | EHV-1 BamHI a & i | EcoR I†-Pme I   | ~2941 BP |
| Fragment 3 | pJF751            | Bam HI-Pvu II   | ~3010 BP |
| Fragment 4 | SPV Hind III M    | Acc I-Hind III  | ~2149 BP |

†Restriction sites introduced by PCR cloning



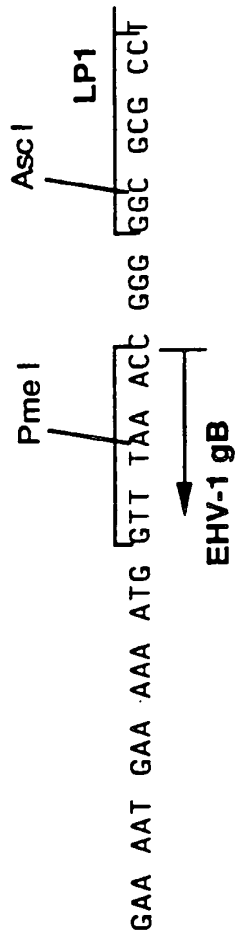
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FIGURE 24C

Junction C



Junction C Continued

LP1

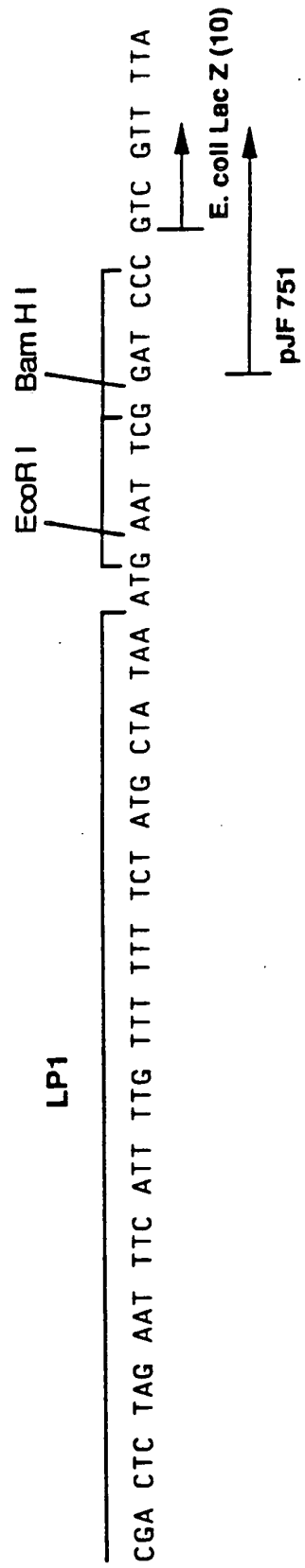


FIGURE 24D

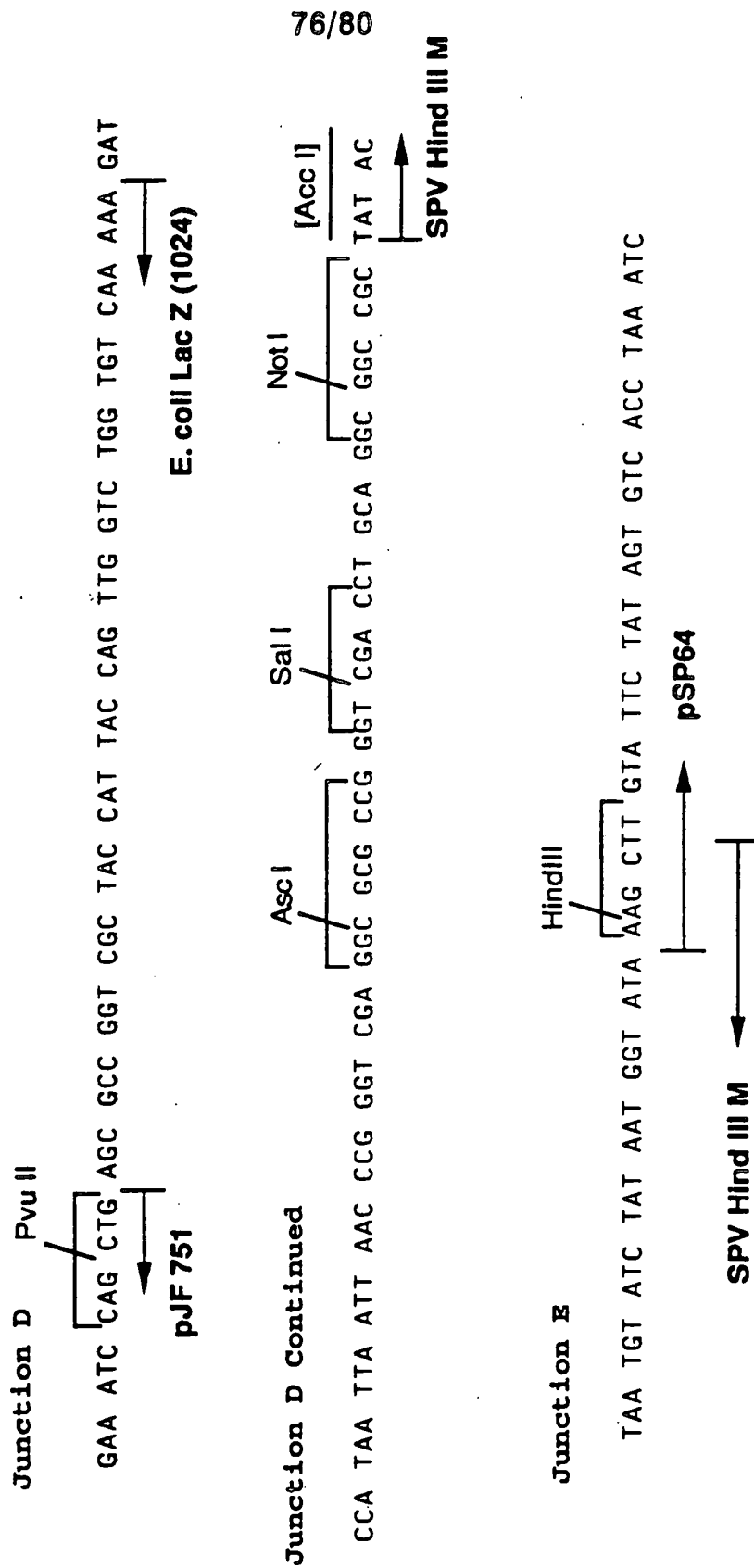
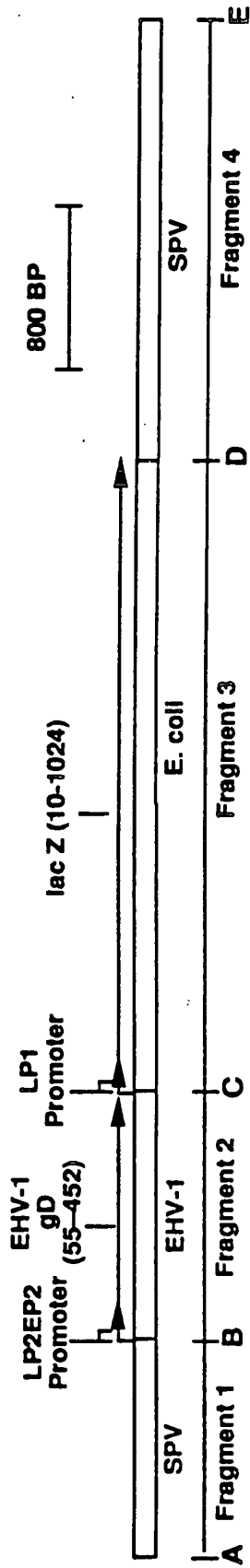




FIGURE 25A  
FIGURE 25B  
FIGURE 25C  
FIGURE 25D

FIGURE 25A

| DNA        | Origin          | Sites             | Size     |
|------------|-----------------|-------------------|----------|
| Vector     | pSP64           | Hind III-Bam HI   | ~2972 BP |
| Fragment 1 | SPV Hind III M  | Bgl II-Acc I      | ~1484 BP |
| Fragment 2 | EHV-1 BamHI "d" | Hind III-Hind III | ~1240 BP |
| Fragment 3 | pJF751          | Bam HI-Pvu II     | ~3010 BP |
| Fragment 4 | SPV Hind III M  | Acc I-Hind III    | ~2149 BP |



ACA GGA AAC AGC TAT GAC CAT GAT TAC GAA TTC GAG CTC GCC CGG GGA TCT

[Bam HI/Bgl II]

SPV Hind III M

pSP64

**[Acc I]**

GTAT AGC GGC CGC CTG CAG GTC GAC TCT AGA TTT TTT TTT TTT TTG GCA TAT AAA

**Not I**

**Sal I**

**Xba I**

**LP2**

**SPV Hind III M**

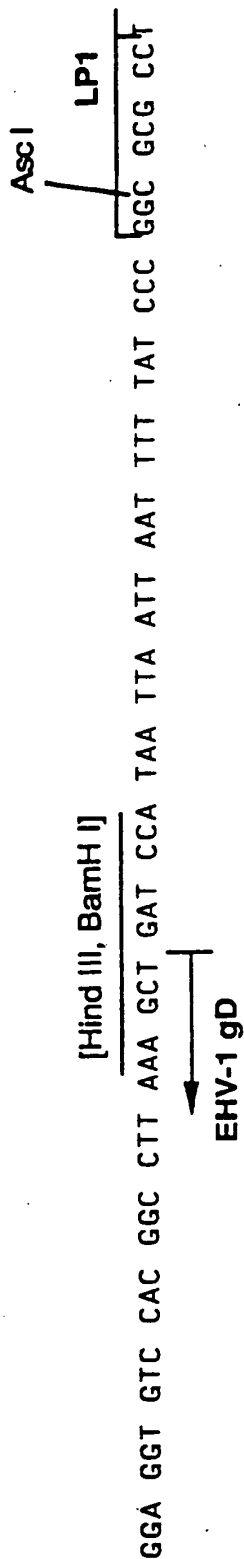
Bgl II  
 EP2  
 TAG ATC TGT ATC CTA AAA TTG AAT TGT AAT TAT CGA TAA TAA AT

EcoR I [BamH I, Hind III]  
G AAT TCG GAT CAG CTT ATG ATG GAT GGA CGT TTG G  
EHV-1 gD (aa 55-452)

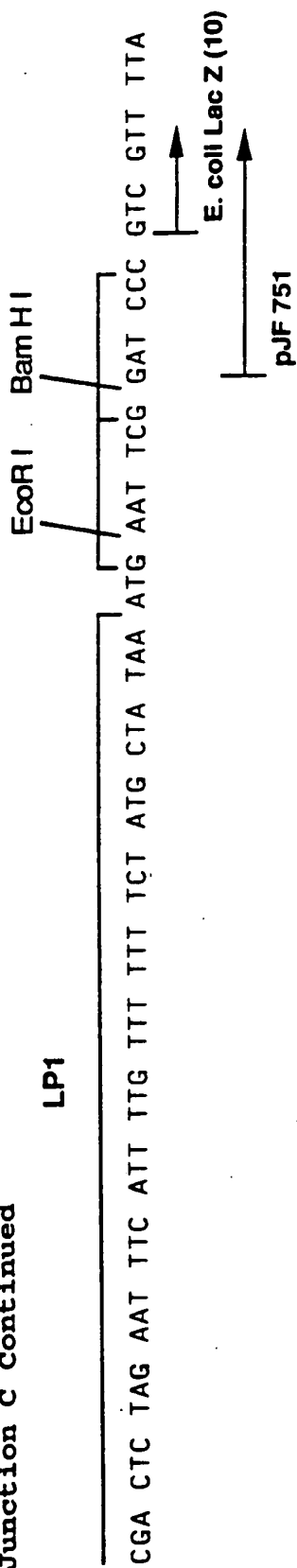
79/80

FIGURE 25C

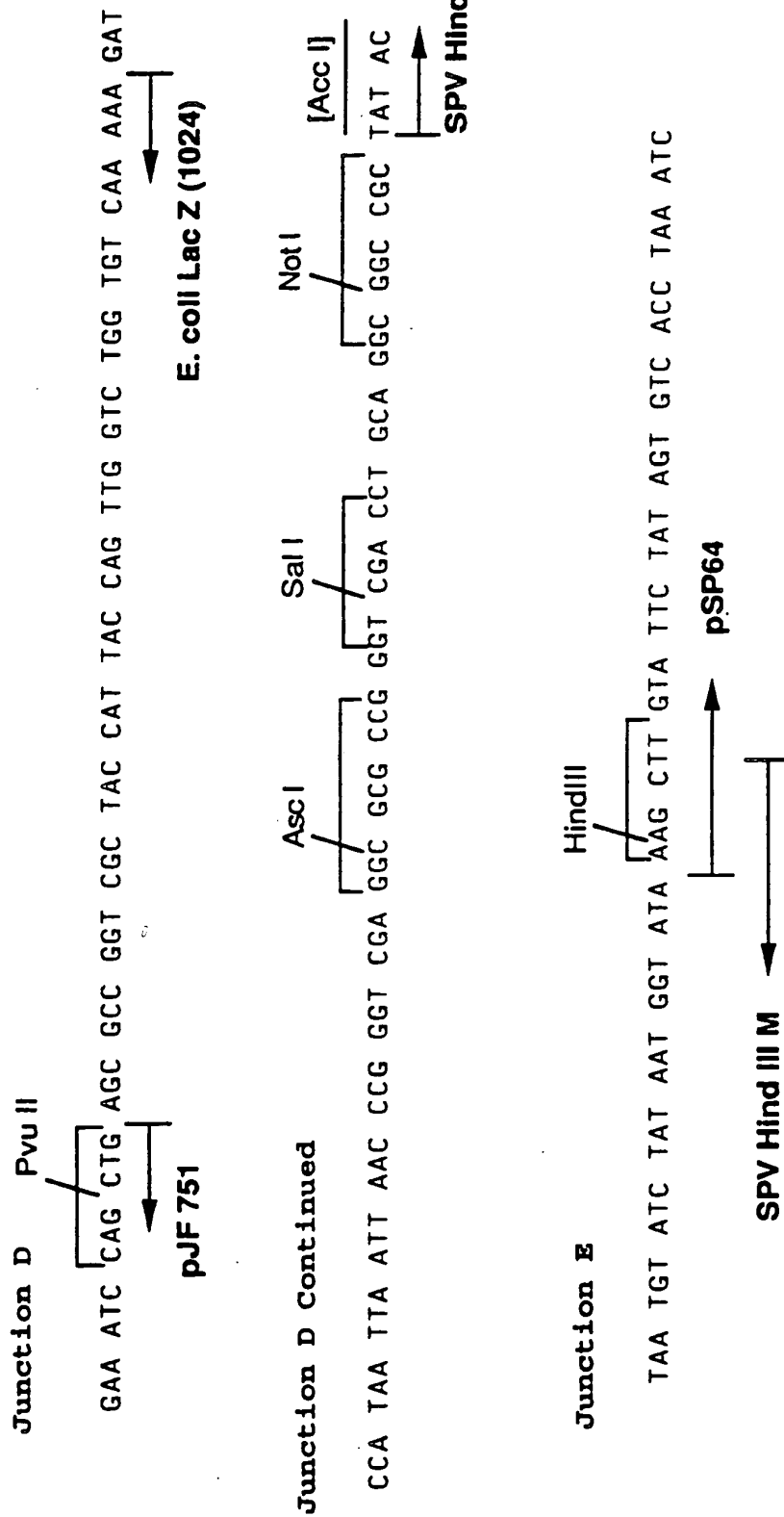
Junction C



Junction C Continued



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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08277

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/275, 39/295, 39/39; C12N 7/01, 15/86

US CL : 424/199.1, 281.1, 93.2; 435/235.1, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/199.1, 281.1, 93.2; 435/235.1, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                         | Relevant to claim No.                                 |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| Y         | WO, A, 93/14194 (COCHRAN ET AL) 22 JULY 1993, see entire document.                                                                                                                                         | 1-50, 52-69, 71-87                                    |
| Y         | Vaccine, Volume 6, issued December 1988, Taylor et al, "Fowlpox virus as a vector in non-avian species", pages 466-468, see entire document, especially abstract and page 467, column 3.                   | 1-50, 52-69, 71-87                                    |
| Y         | Critical Reviews in Immunology, Volume 10, Issue 1, issued 1990, Tartaglia et al, "Poxvirus-Based Vectors as Vaccine Candidates", pages 13-30, see entire document, especially page 23 and tables 3 and 4. | 1-5, 10-34, 40-50, 52, 53, 56-59, 62-69, 71-77, 79-85 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

|                                                                                                                                                                        |   |                                                                                                                                                                                                                                              |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                               | T | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| *A document defining the general state of the art which is not considered to be of particular relevance                                                                | X | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| *E earlier document published on or after the international filing date                                                                                                | Y | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | g | document member of the same patent family                                                                                                                                                                                                    |
| *O document referring to an oral disclosure, use, exhibition or other means                                                                                            |   |                                                                                                                                                                                                                                              |
| *P document published prior to the international filing date but later than the priority date claimed                                                                  |   |                                                                                                                                                                                                                                              |

Date of the actual completion of the international search

21 OCTOBER 1994

Date of mailing of the international search report

04 NOV 1994

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/08277

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                           | Relevant to claim No.                                |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Y         | Trends in Biotechnology, Volume 10, issued December 1992, Ramshaw et al, "Cytokine expression by recombinant viruses - a new vaccine strategy", pages 424-426, see entire document.                                                                                                                          | 6-9, 40-50, 54, 55, 62-69, 87                        |
| Y         | US, A, 4,920,213 (DALE ET AL) 24 APRIL 1990, see entire document, especially abstract and columns 21-25.                                                                                                                                                                                                     | 10-16, 40-50, 56, 57, 62-69, 72, 73, 80, 81          |
| Y         | Journal of General Virology, Volume 70, issued 1989, Whalley et al, "Identification and Nucleotide Sequence of a Gene in Equine Herpesvirus 1 Analogous to the Herpes Simplex Virus Gene Encoding the Major Envelope Glycoprotein gB", pages 383-394, entire document, especially page 392, final paragraph. | 10-12, 17, 18, 40-50, 56, 57, 62-69, 72, 74, 80, 82  |
| Y         | WO, A, 92/02252 (O'CALLAGHAN) 20 FEBRUARY 1992, see entire document.                                                                                                                                                                                                                                         | 10, 11, 19, 20, 40-50, 56, 57, 62-69, 72, 74, 80, 82 |
| Y,P       | Journal of Dairy Science, Volume 76, No. 8, issued 1993, Yancey, "Recent Advances in Bovine Vaccine Technology", pages 2418-2436, see pages 2419-2422 and pages 2428-2430.                                                                                                                                   | 21-37, 40-50, 58, 59, 62-69, 75-77, 83-85            |
| Y         | WO, A, 92/07940 (SAMAL) 14 MAY 1992, see entire document.                                                                                                                                                                                                                                                    | 21-28, 40-50, 58, 59, 62-69, 75, 83                  |
| Y         | US, A, 4,847,081 (RICE) 11 JULY 1989, see entire document, especially claim 3.                                                                                                                                                                                                                               | 21, 22, 29-31, 40-50, 58, 59, 52-69, 76, 84          |
| Y,P       | WO, A, 94/11521 (KOWALSKI ET AL) 26 MAY 1994, see pages 17 and 38-40.                                                                                                                                                                                                                                        | 32-34, 77, 85                                        |
| Y         | Archives of Virology, Volume 131, issued 1993, Heine et al, "Infectious bursal disease virus structural protein VP2 expressed by a fowlpox virus recombinant confers protection against disease in chickens", pages 277-292, see entire document.                                                            | 35-39, 40-50, 60-69, 78, 86                          |
| A         | Journal of Biological Chemistry, Volume 266, Number 3, issued 25 January 1991, Moss et al, "Cytoplasmic Transcription System Encoded by Vaccinia Virus", pages 1355-1358, see entire document.                                                                                                               | 48-51, 67-70                                         |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/08277

### B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Derwent Biotechnology Abstracts, Medline, APS. Keywords: recombinant, swinepox?, suipox?, swine, pox?, vaccinia, il2, cytokine#, interleukin#, tumor, antigen, antigens, equine influenza, neuraminidase, equine(w)herpes?, gb, glycoprotein b, gd, glycoprotein d, cattle, cow, cows, bovine, rsv, syncytial, parainfluenza, diarrhea, virus, viral, 48, gp48, 53, gp53, bursal, ibdv, cmv, cytomegalovirus, promoter?, transcription.